HAEMOLYTIC STREPTOCOCCAL INFECTIONS

AND ACUTE RHEUMATISM

by

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GENERAL INTRODUCTION

In temperate climes the haemolytic streptococcus is the most important excitant of acute infection in man. The upper respiratory passages form a natural habitat for the commensal existence of the organism, and as a consequence the respiratory tract bears the brunt of pathogenic action. Furthermore, the presence of the agent in the naso-pharyngeal secretions leads to its widespread dispersal by means of droplets. Many are the disease patterns attributable to the haemolytic streptococcus for, in addition to its confirmed activity in scarlatina, erysipelas, puerperal fever, wound infection and epidemic tonsilitis, the organism may invade practically every system in the body, and few tissues are immune against attack.

Knowledge of the methods whereby the haemolytic streptococcus produces infection, both in the individual and throughout a community, has been steadily amplified but is far from complete. Outstanding among the points upon which finality has not been reached is the association between haemolytic streptococcal infection and acute rheumatism. As will be shown later, this linkage was first based on

clinical observations and has since been supplemented by laboratory investigations. Its general acceptance has been prevented by several considerations, such as the differences in pathology exhibited by the pyogenic lesions due to Str. haemolyticus and those of acute rheumatism.

Economically, rheumatism must now rival tuberculosis as the infective disease which most seriously impairs the efficiency of this country. The extent of the problem may be gathered from the fact reported by Bach et al.(1939) that the London County Council alone expends almost £ 250,000 annually on the prevention and treatment of juvenile rheumatism. Whereas vigorous countermeasures have produced a steady decline in the incidence of tuberculosis, it is extremely unlikely that a parallel reduction in rheumatism has occurred. It is a regrettable omission in public health administration that acute rheumatic fever is not a notifiable disease, so that the approximate state of affairs can only be deduced from returns issued by sections of the community in which the incidence is recorded. These indicate that the severity of the disease, and the tendency for it to be followed by carditis and chronic rheumatism, are on the increase. Thus the latest available figures for the Royal Navy include the following invaliding rates and deaths due

to acute rheumatism:-

YEAR	INVALIDED	DEATHS
1934	18	3
1935	29	4
1936	70	2

Surveying the end-results in a series of 300 cases of juvenile rheumatism, Wallace (1937) recorded that 25 per cent of all rheumatic children died from heart disease before they left school, and a further 30 per cent were crippled for life. This does not appear to be a local excess for Bach et. al. (1939) stated that, in the London area, 20 per cent of rheumatic children were fit only for light employment when they left school. Davidson and Duthie (1938) have shown that of the total population of 5,000,000 in Scotland, 50,000 insured persons were wholly incapacitated each year for an average of 60 days. They estimated that there must be approximately 335,000 new cases of rheumatism each year in Scotland, of which 26,000 were cases of acute or subacute rheumatic fever. This condition produces its maximum effect in childhood and adolescence. Thus Hedley (1940) , has recorded that 63 per cent of cases were under 14 years of age in a series of 2539 cases of rheumatic fever admitted to hospitals in Philadelphia from 1930 to 1934. If all these facts can be accepted for general application, then it is deduced that in Scotland alone there are

approximately 3000 deaths per annum in school children as a result of acute rheumatism, and the same number leave school as cardiac cripples. These facts have been selected from many which emphasise the gravity of the situation. This trend will probably continue and should acute rheumatism finally prove to be yet another manifestation of haemolytic streptococcal infection, then the group of diseases due to this organism will undoubtedly constitute the greatest of all future problems in preventive medicine.

The research work presented in the thesis has therefore been directed toward haemolytic streptococcal infections, particularly with reference to the development of acute rheumatism. Because of the controversial nature of our knowledge on various points, it has been necessary to proceed in some sections on the lines of previous investigations, repeating these in large groups with adequate controls in order to reach a significant conclusion. Original observations, which have since received independent confirmation, are also included. Each section of the thesis has been treated as a separate problem with conclusions based on the experimental or epidemiological observations made. Several sections have already appeared in published form, including the following papers :-

1. Observation on the toxic fractions of scarlatinal streptococci.

Green, C.A. 1935. J.Hyg. XXXV. 93.

2. The serological types of haemolytic streptococci in epidemic scarlatina.

Green, C.A. 1937. J.Hyg. XXXVII. 318.

3. The serological examination of haemolytic streptococci in acute rheumatic and control groups.

Green, C.A. 1938. B.M.J..I. 1147.

4. Sensitivity of rheumatic subjects to streptococcal products.

Green, C.A. 1938. J. Path.Bact., 1938. XLVII. 337.

5. Rheumatic carditis: post-mortem examination of nine consecutive cases.

Green, C.A. 1939. Ann.Rheum.Dis.I. 86.

6. Some observations on the possible streptococcal aetiology of acute rheumatism.

Green, C.A. 1939. H.Royal Naval Med.Service.XXV.218.

7. The formol-gel reaction and erythrocyte sedimentation rate in acute rheumatism.

Green, C.A., Thomson, S., and Glazebrook, A.J. 1939. Ann. Rheum. Dis. I,180.

8. Preliminary observations on the use of convalescent serum in the treatment of acute rheumatism.

Green, C.A., Glazebrook, A.J., Thomson, S. and Hopkins, W.A. 1940. Proc. Roy. Soc. Med. XXXIII.275.

9. Reactions induced by intradermal injection of rheumatic joint fluid: neutralisation by convalescent sera.

Green, C.A. 1940. Proc. Roy. Soc. Med. XXXIII.421.

10. Antistreptolysin O titres in various groups.

Green, C.A. In press.

11. Observations on the antistreptolysin O titre in relation to the mechanism of acute rheumatism.

Green, C.A. In press.

12. Effect of prontosil on the antistreptolysin O titre in rabbits during immunisation.

Green, C.A. In press.

13. Epidemiology of haemolytic streptococcal infection in relation to acute rheumatism.

Green, C.A. In press.

In the final summary, the various sections are discussed in relation to each other and to certain concepts in regard to the aetiology of acute rheumatism.

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SECTION A

THE SEROLOGICAL TYPES OF HAEMOLYTIC STREPTOCOCCI IN EPIDEMIC SCARLATINA

INTRODUCTION

Scarlet fever is one of the group of acute infectious diseases which, in distribution, may occur as sporadic cases or as epidemics of varying extent. Edinburgh and its surrounding district was visited in 1933 by an epidemic which rapidly assumed considerable proportions. The onset of the epidemic was presaged by an unusual rise in the number of cases during the month of May. It so happened that during the preceding two months of March and April, a large series of strains of haemolytic streptococci from acute scarlatina had been isolated for the purpose of carrying out an investigation on toxin production. The collection of strains was continued during the epidemic and a unique opportunity was therefore presented of studying the characters of strains isolated during an immediate pre-epidemic period of two months together with those of the epidemic itself.

Of the various methods devised in the attempted classification of haemolytic streptococci, that which has been most extensively used has been the agglutination reaction. Yet the results obtained by

different workers have often been at variance. Moser and von Pirquet (1902), utilizing direct agglutination reactions with sera from scarlet-fever patients and also from horses immunized with haemolytic streptococci isolated from the blood of fatal cases of scarlatina, concluded that scarlet-fever strains formed a group serologically distinct from those isolated from other diseases. In this respect he received confirmation by Meyer (1902) and by Rossiwall and Schick (1905). On the other hand, Aronson (1903), using the same technique as that followed by Moser and von Pirquet, was unable to corroberate their findings, while Neufeld (1903), using immune rabbit sera , also failed to define a specific scarlatinal group. For a prolonged period no further results were reported until with the introduction of new methods, an impetus was given to further study of the subject. By the method of agglutinin-absorption Tunnicliff (1920), Bliss (1920) , and Gordon (1921) each found that at least 80 per cent of scarlatinal strains were of one serological type, but Williams (1924) was again able to demonstrate multiplicity of types, of which the largest constituted only 15 per cent of all strains examined. Williams showed further that strains identical with those from scarlatina were recoverable from presumably non-scarlatinal sources such as cases of puerperal fever and erysipelas. This, too, has received ample confirmation at the hands of Smith (1926), (1927), James (1926) and MacLachlan and Mackie (1928). An extensive investigation of this

nature was reported by Griffith (1927), who found that 60 per cent of strains from acute scarlatina fell into one or other of types 1,2,3 and 4 of his classification which now comprises twenty-nine types (Griffith, 1934). Andrewes and Christie (1932) by careful serological analysis were able to identify three of these types, while Allison and Gunn (1932) have confirmed the results in full. No reports have as yet appeared concerning the serological investigation of strains from an epidemic of the magnitude of that experienced in Edinburgh in 1933, but Glover and Griffith (1931), Griffith (1934) and Keogh et al. (1939) have found that in small epidemics there is usually a preponderance of a single type.

Previous reports differ also as to the incidence of carriers of haemolytic streptococci among scarlatinal convalescents. Williams (1924) noted that after 30 days only 20 per cent of convalescents were carriers and in these the numbers of streptococci were few. Kirkbride and Wheeler (1930) found that between 50 and 60 per cent of convalescents were still carriers at the same period. Similarly, Gunn and Griffith (1928) record a carrier rate of 49 per cent, while Brown and Allison (1935) found that 82.8 per cent of patients, irrespective of their length of hospitalization, had haemolytic streptococci in the throat or nose on discharge, and suggest that the true percentage of carriers was probably higher, since return cases followed the discharge of a certain number of

those giving a negative cultural result. Of more importance from the point of view of dissemination by carriers is the fact that Kirkbride and Wheeler (1930) could find no fundamental difference as regards the pathogenicity or virulence between strains isolated from the acute and convalescent phases, respectively, of illness. Yet only two of thirty-four carriers in their series were reported to have become infecting cases. Not one single return case was ascribed to the 49 per cent of discharge carriers in Gunn and Griffiths' series, and this was attributed by the authors to the probably early abolition of the carrier state induced by the altered environment of home conditions. Brown and Allison (1935) made the observation that the degree of infection on discharge as indicated by cultural methods was correlated with the return-case rate, which was 3 per cent in mild and moderate, but 6 per cent in heavy or very heavy infection.

The present study was undertaken in order to re-examine the questions referred to above and also to obtain if possible some information regarding the factors which determine the onset of a widespread epidemic in a community subjected apparently to the same risks as prevail in non-epidemic times.

METHODS

Isolation of strains

On admission to hospital a throat swab was taken from each patient and used to inoculate a 5 per cent rabbit-blood agar plate. This plate was then

incubated aerobically at 37°C. for 24 hours. A single colony showing beta lysis was emulsified in 0.5 per cent phosphate broth from which were inoculated two tubes, one containing 5 c.c. of 0.5 per cent phosphate broth and the other 5 c.c. of 0.5 per cent glucose broth. The serological examination of the particular strain was initiated by using the growth in phosphate broth after 24 hours incubation at 37°C., while the 24 hours' glucose broth culture was used for inoculating a further 200 c.c. glucose broth if the toxin production of the strain was also to be examined.

On discharge from hospital a second throat swab and, in addition, a nasal swab were again examined in the manner described above. From many of the patients further swabs were examined during the course of their stay in hospital.

Preparation of antisera.

Initially four rabbit antisera were prepared by the intravenous injection of heat killed 24-hour broth cultures of types 1,2,3 and 4 strains of Streptococcus haemolyticus supplied by Dr.Griffith. It was found that although the sera so prepared agglutinthe homologous strains to high titre, a large proportion of the locally isolated strains were agglutinated, if at all, by only low dilutions of these sera. Accordingly additional sera were prepared against several of these strains and finally eight were utilized, four for Griffith's types 1,2,3 and 4 and four for types provisionally named A,B,C and D, one of these

A, being serologically identical with type 5.

Direct agglutination reaction

The cultures derived from each case were in the first instance tested for direct agglutination by the eight type specific sera, the method described by Smith (1926) being followed.

Agglutinin absorption

In view of the frequency with which co-agglutination occurred in many strains, the method of agglutinin absorption was used to supplement direct agglutination results. A strain was accepted as belonging to one of the eight types if it absorbed completely from its antiserum the homologous agglutinins.

EXPERIMENTAL OBSERVATIONS

Quantitative results of examinations on admission

Single throat swabs from 1875 acute cases were examined for the presence of haemolytic streptococci and of these, 1581 or 84.3 per cent were found positive, while 15.7 per cent were negative or yielded no growth. The detailed quantitative results are given in Table 1 (p. 13).

For comparative purposes the results of plating each admission swab were designated +, ++ or +++, according to the number of colonies of haemolytic streptococci obtained. Although it was not assumed that this division of positive results into groups, according to the number of colonies appearing on the plates, afforded an absolute measure of the degree of infection, yet it was taken to give some relative

SECTION A TABLES I and II

Table I. *Results of examination of admission throat swabs from acute scarlatina, grouped according to number of haemolytic streptococcal colonies on inoculated plate*

\+ =less than 10 colonies per plate.
\+ + =between 10 and 20 colonies per plate.
\+ + + =more than 20 colonies per plate.
0=no growth on plate.
– =no haemolytic streptococci detectable on plate.

	Throat swab plate				
Month of admission	+	+ +	+ + +	–	0
Mar.	12	21	41	8	0
Apr.	18	45	30	24	3
May	24	69	87	18	0
June	28	46	108	22	2
July	29	109	117	21	5
Aug.	32	77	102	16	5
Sept.	40	198	76	107	0
Oct.	45	111	49	41	1
Nov., Dec., Jan.	10	13	44	12	9
Total	238	689	654	269	25
%	12·69	36·74	34·77	14·34	1·32

84·3% positive; 15·7% negative

Table II. *Results of examination of discharge throat swabs, grouped according to number of haemolytic streptococcal colonies on inoculated plate*

\+ =less than 10 colonies per plate.
\+ + =between 10 and 20 colonies per plate.
\+ + + =more than 20 colonies per plate.
0=no growth on plate.
– =no haemolytic streptococci detectable on plate.

Results of admission examination		Discharged without examination	Examined on discharge	Discharge swab results			
				+	+ +	+ + +	–
Positive	+238	90	148	20	19	9	100
			100%	13·5%	12·8%	6·1%	67·6%
„	+ +689	233	456	85	75	21	275
			100%	18·6%	16·4%	4·6%	60·3%
„	+ + +654	196	458	78	68	24	288
			100%	17·0%	14·9%	5·2%	62·8%
Negative	294	106	188	27	27	9	125
No swab on admission	298	0	298	49	38	21	190
		Total	1548	259	227	84	978
		%	100	16·7	14·7	5·4	63·2

indication of the number of haemolytic streptococci present. Results obtained by this method naturally tended to err on the side of revealing a lower estimate of the degree of infection than was actually present. Therefore the absence of haemolytic streptococci in 15.7 per cent of the primary plates by no means signified their absence from the throat, but rather failure to isolate on account of technical difficulties such as the overgrowth of haemolytic streptococci by other organisms, variations in the methods of swabbing resulting in differences in the amount of inoculum, and the local use of antiseptics before the taking of a swab.

Quantitative results of examinations on discharge

As the number of swabs dealt with at one time was limited by such conditions as the amount of time and media available it was found impossible to examine on discharge all those cases examined on admission. However, the examination of 1062 of the cases yielding positive swabs on admission was completed, and to these were added 188 cases whose admission swabs were negative and 298 cases which were not examined on admission, the total number of convalescents being 1548. Of these the throat swabs from 978 were found negative while 570 or 36.8 per cent were positive. Table II (p.13) indicates the numbers of haemolytic streptococci recovered from swabs on discharge and for comparison these are grouped according to the results

on admission. It will be seen that although there were 36.8 per cent of carriers on discharge, only 20.1 per cent yielded more than 10 colonies per plate, i.e. ++ and +++ groups, as contrasted with the 71.5 per cent of acute cases showing a similar degree of infection. There was no significant difference as to the incidence of carriers among the +,++ and +++ admission groups, the carrier rate on discharge being 32.4,39.6 and 37.1 per cent respectively (Table II, P.13) nor did the proportion of +,++ and +++ results in each of these groups at the time of discharge vary to any marked extent.

Serological examination of strains isolated during the early stage of illness

By the methods described above there were defined eight serological types of which four corresponded to Griffith's types 1,2,3 and 4. Representative strains of the remaining four types A,B,C and D have since been submitted to Dr.Allison who reported that type A was identical with Griffith's type 5, but that types B,C and D were not represented in the series. In this communication type A will hereafter be referred to as type 5.

It was found possible to place 94.1 per cent of the strains isolated on admission into one or other of these types, leaving a residue of 5.9 per cent which either reacted to none of these type sera or failed to yield suitable suspensions for agglutination. Auto-agglutination is a well recognised

technical difficulty encountered in the serological investigation of haemolytic streptococci and, in this inquiry, the special methods used reduced the proportion of strains which had to be discarded as unsuitable to 2.3 per cent.

The number of admission strains of each type isolated during successive months of the year are indicated in Table III (p.17). From this table it will be noted that there was, over the whole period, a striking preponderance of type 5 cases. Thus 47.8 per cent of all cases were due to this type whereas Griffith's types constituted 29.3 per cent of the total i.e. Combined figure for types 1,2,3 and 4 . Reference to Table IV(p.17) which indicates the proportion of all notified cases of scarlatina subjected to serological examination shows that in any month from April to August the data at hand referred to at least 75 per cent of such notifications, and such data may reasonably be taken to afford information applicable to the epidemic as a whole. During March and September the proportion of cases examined was not so large but was above 50 per cent. With this in mind a study of the proportion of acute cases due to the various types in each particular month reveals several interesting facts (Table V, p.17). Thus in March, two months before the onset of the epidemic, there was already a marked preponderance of type 5 cases in those admitted to hospital. During that month type 5 cases comprised 43.24 per cent of the total, the next

SECTION A TABLES III, IV and V

Table III. *Serological types of haemolytic streptococci from admission throat swabs in acute scarlatina*

Month of admission	Type 1	2	3	4	5	B	C	D	Total typed	Total not typed	No. cases
Mar.	7	6	17	7	32	2	0	1	72	2	74
Apr.	10	10	20	6	38	4	1	1	90	3	93
May	10	9	21	11	105	10	4	5	175	5	180
June	11	8	8	0	143	3	1	1	175	7	182
July	12	8	29	12	135	18	15	5	234	21	255
Aug.	11	8	34	8	115	12	4	4	196	15	211
Sept.	19	12	60	10	133	13	31	12	290	24	314
Oct.	30	26	43	29	41	7	21	0	197	8	205
Nov., Dec., Jan.	7	5	16	2	14	3	8	4	59	8	67
Total	117	92	248	85	756	72	85	33	1488	93	1581
%	7·4	5·8	15·7	5·4	47·8	4·6	5·4	2·1	94·1	5·9	100

Table IV. *Number of cases of acute scarlatina bacteriologically examined as compared with the total number of notified cases in Edinburgh during the year* 1933

Month of admission	Number of notifications	Number examined	Percentage of notifications
Jan.	100	0	0
Feb.	79	0	0
Mar.	127	74	58·28
Apr.	124	93	75·00
May	226	180	79·64
June	228	182	79·82
July	308	255	82·79
Aug.	242	211	86·71
Sept.	468	314	67·1
Oct.	924	205	22·18
Nov.	942	53	5·62
Dec.	748	14	1·87
Total	4516	1581	

Table V. *Variation in number of cases of acute scarlatina due to the various serological types of haemolytic streptococci, expressed as percentages of the total number of cases examined during the month.*

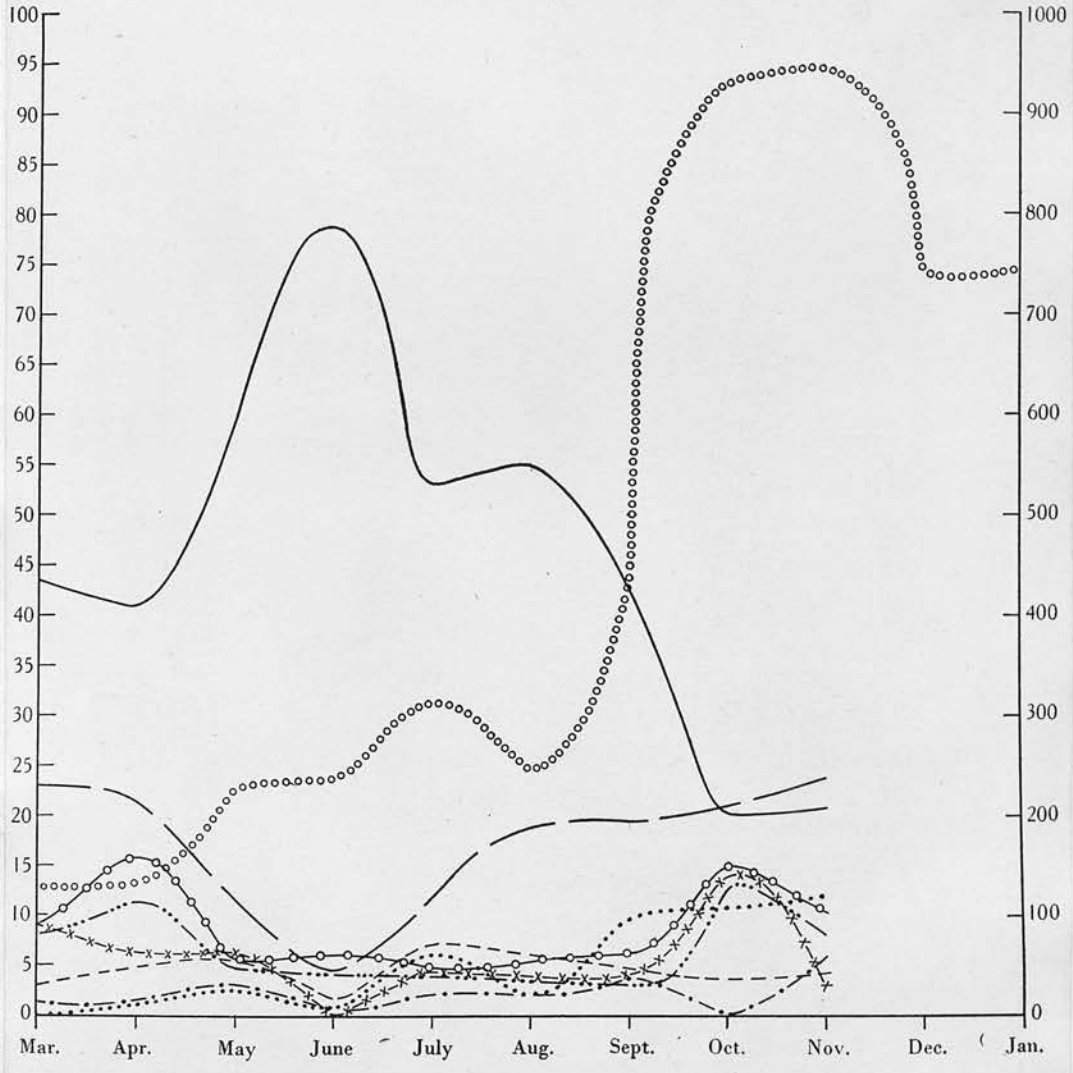
Month of admission	Serological type 1	2	3	4	5	B	C	D	Un-typed
Mar.	9·4	8·1	22·9	9·4	43·2	2·7	0·0	1·3	2·7
Apr.	10·7	10·7	21·5	6·4	40·8	4·3	1·1	1·1	3·2
May	5·5	4·9	11·6	6·11	58·3	5·5	2·2	2·7	2·7
June	6·0	4·4	4·4	0·0	78·5	1·6	0·5	0·5	3·8
July	4·6	3·1	11·3	4·6	52·9	7·0	5·8	1·9	8·2
Aug.	5·2	3·8	18·8	3·8	54·7	5·7	1·9	1·9	6·6
Sept.	6·0	3·8	19·1	3·1	42·3	4·1	9·8	3·8	7·6
Oct.	14·7	12·8	21·1	14·2	20·9	3·4	10·3	0·0	2·9
Nov., Dec., Jan.	10·4	7·4	12·7	2·9	20·8	4·4	11·9	5·9	11·9

largest group of 22.97 per cent being type 3 in origin. During the month of April the proportion of type 5 cases remained approximately the same. In May, with the rise of the epidemic the proportion of type 5 cases mounted to 58.3 per cent and in June had reached 78.5 per cent. From this peak period the proportion of type 5 cases fell during the next three months to 42.3 per cent, but, in the same months, the absolute number of type 5 cases remained approximately the same. This fall in the proportion of type 5 cases was largely compensated for by a rise in the number of type 3 strains(Graph 1, p.19).

Serological examination of discharge strains

Of the 1581 cases examined on admission, throat and nasal swabs were taken on discharge from 1062 persons and of these 625 or 58.9 per cent yielded no haemolytic streptococci(Table VI, p. 21). This figure approximated closely to 63.2 per cent of negative swabs noted in the extended survey of discharged cases(Table II, p.13). The 41.1 per cent carrier group comprised 31.5 per cent in whom the type on admission was identical with that isolated on discharge, 3.4 per cent whose discharge swab revealed an additional type together with the admission type, and 6.2 per cent in whom the admission type was absent and replaced by some other type. Thus in 34.9 per cent the admission type was still present in the throat or nose at the time of discharge. Table VII(p.21)continues this analysis further and shows the combined

SECTION A GRAPH I



Percentage of all cases examined in each month due to:

Type 1 ——o——o——o—— Type 2 ——···——···—— Type 3 ———— ————

Type 4 ——x——x——x—— Type 5 ———————— Type B - - - - - - - -

Type C ····· Type D —·—·—·—

Number of notifications of Scarlatina in each month: ooooooooooo

Graph I. Showing the monthly variation in the percentage number of cases of acute scarlatina due to various serological types of haemolytic streptococci, during the year 1933.

results on discharge grouped according to the type on admission. The numbers of positive results on discharge in the type 5 and type 3 admission groups were sufficiently large to yield significant data, and it will be seen that the same types were isolated on discharge, either alone or together with some other type, in 85.1 and 82.9 per cent of these groups respectively. The numbers in the other groups were too small to be expressed as percentages but they all approximated to those in the larger groups. Further, 22.6 per cent of type 5 cases and 28.9 per cent of type 3 cases yielded an additional type on discharge.

In Table VIII (p.21) are presented the total numbers of the additional types found in throat swabs on discharge in the various admission groups. Of the 103 strains thus isolated, 63 or 61.1 per cent failed to be agglutinated by anyof the eight type sera, 17 or 16.5 per cent were type 5 and 15 or 14.5 per cent were type 3, the remaining types constituting only small percentages of the total.

Thus the high carrier rate was, in the main, due to the persistence of the admission types. There was, in addition, a tendency apparently for the predominating types, 5 and 3, to spread to convalescents recovering from infection by other types. This latter finding alone could not be accepted as evidence of any enhanced spreading property on the part of these two types since the high concentration of type

SECTION A TABLES VI, VII and VIII

Table VI. *Results of examination of discharge throat and nasal swabs grouped according to persistence or otherwise of serological type of haemolytic streptococcus isolated on admission*

Month of admission	Positive admission		Discharge swab results									
			No haemolytic streptococci		Admission type only		Admission type + additional type		Additional type only		No discharge examination	
	Typed	Untyped										
Mar.	72	2	32	1	14	0	3	0	11	1	12	0
Apr.	90	3	35	1	31	0	2	0	13	2	8	0
May	175	5	94	3	40	1	6	1	12	0	23	0
June	175	7	71	2	27	1	4	0	4	2	69	2
July	234	21	117	7	58	3	5	0	11	3	43	8
Aug.	196	15	95	6	53	4	8	0	0	0	40	5
Sept.	290	24	100	8	66	4	6	0	5	0	113	12
Oct.	197	8	46	5	28	0	1	1	4	0	118	2
Nov., Dec., Jan.	59	8	1	1	1	1	0	0	0	0	57	6
	1488	93	591	34	318	16	35	2	60	6	484	35
Total admissions	1581		625		334		37		66		519	
No discharge examination	519											
	1062		58·9 %		31·5 %		3·4 %		6·2 %			

Table VII. *Results of examination of discharge throat and nasal swabs, grouped according to type on admission*

Admission examination		No discharge examination	Discharge examination									
			(I) No haemolytic streptococci		(II) Admission type only		(III) Admission + different type		(IV) Different type only			
Type	Total		Total	%	Total	%	Total	%	Total	%	Total	%
A (5)	756	236	317	60·9	157	77·2	16	7·8	30	14·7	203	39·1
B	72	18	30	55·5	22	91·7	1	4·2	1	4·2	24	44·4
C	85	34	30	58·8	15	71·4	2	9·5	4	19·1	21	41·2
D	33	8	20	80·0	5	100·0	0	0	0	0	5	20·0
1	117	33	51	60·7	26	86·5	2	6·6	5	16·6	33	39·3
2	92	32	32	53·3	22	78·5	4	14·2	2	7·1	28	46·6
3	248	85	87	53·3	54	71·1	9	11·8	13	17·1	76	46·6
4	85	38	24	51·1	17	73·9	1	4·3	5	21·7	23	48·9
Untyped	93	35	34	58·9	16	65·2	2	8·7	6	26·1	24	41·1
Total	1581	519	625		334		37		66		437	

Table VIII. *Numbers of serological types of haemolytic streptococci, other than those present on admission, isolated from discharge throat swabs*

Type on admission	Number of additional discharge types								Untypable
	1	2	3	4	5	B	C	D	
A (5)	1	1	10	—	—	—	—	—	33
B	—	—	—	—	—	—	—	—	2
C	—	—	1	—	1	1	—	—	3
D	—	—	—	—	—	—	—	—	—
1	—	—	—	1	4	—	—	—	2
2	—	—	—	—	2	—	—	—	4
3	1	1	—	1	4	—	—	—	14
4	—	—	1	—	—	—	—	—	5
Untypable	—	—	2	—	6	—	—	—	—
Total	2	2	14	2	17	1	0	0	63

5 and type 3 cases in the wards during the epidemic would inevitably result in increased chance of cross infection due to these types.

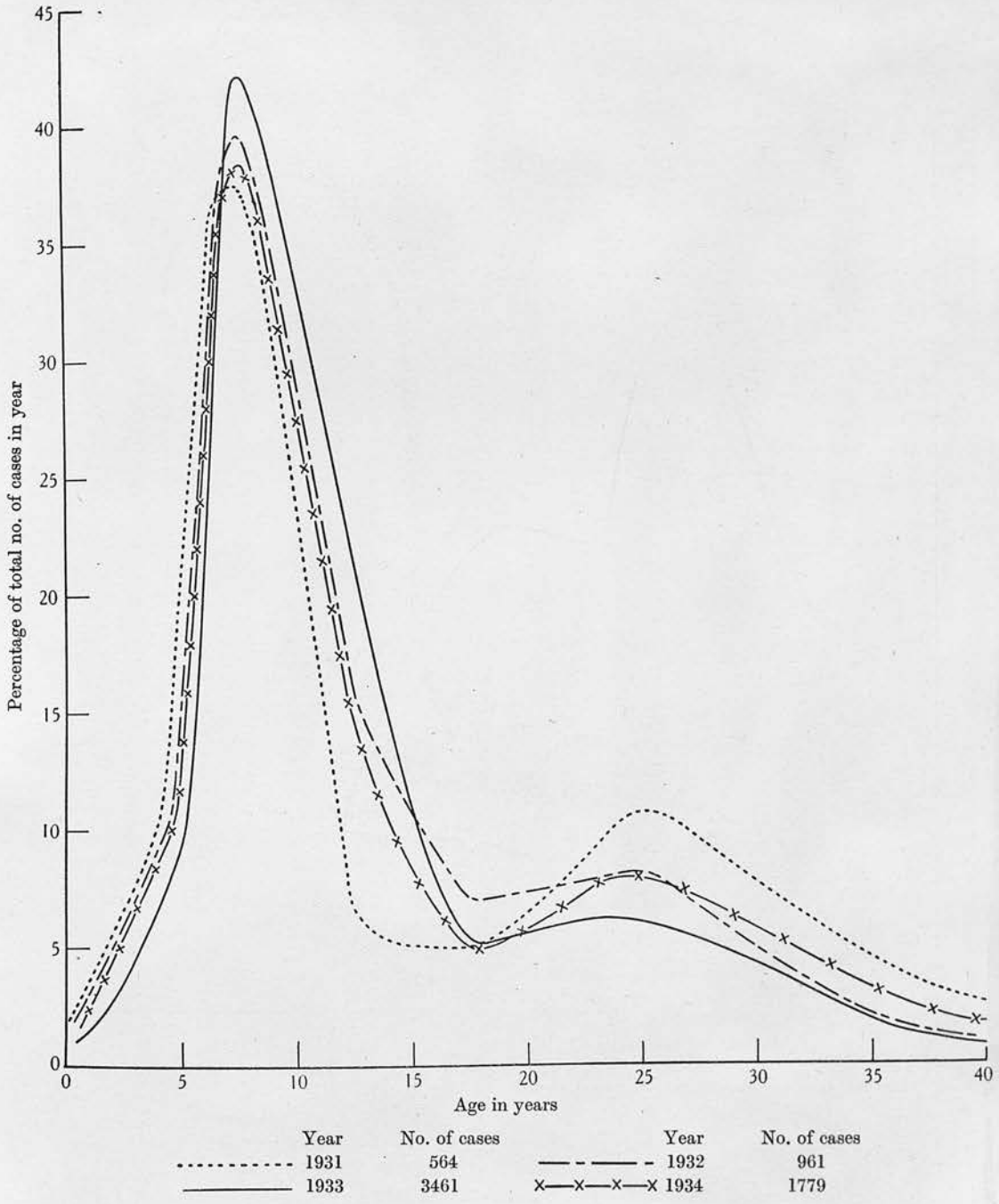
Non-agglutinable discharge strains

Among the additional strains isolated on discharge was a large group of 61.1 per cent which apparently did not belong to any of the eight common types. There was the possibility that certain of these may really have represented strains which, owing to continued growth in the secretions of persons undergoing immunization during convalescence, had lost type specificity. An attempt was made, by mouse passage, to raise the virulence of six such strains in the hope that type characters might appear. Each strain was passed, by intraperitoneal injection, through a series of six mice, with intermediate plating on rabbit-blood agar between each passage. The cultures derived from the final passage were then tested against the specific sera but again failed to conform to any of the types.

Age incidence

Graph11 (p.23) demonstrates that the majority of cases occurred between the ages of 5 and 15 years. A low incidence at all ages up to 5 years was followed by a very sharp rise in the curve till the peak was reached between 5 and 10. Thereafter the decline in incidence with increase in age was rapid but regular, there being a small rise between 20 and 25. The

SECTION A GRAPH II



Graph II. Showing the age group incidence, expressed as a percentage of the yearly number of cases of acute scarlatina in Edinburgh during the period 1931–1934.

corresponding data for the years 1931, 1932 and 1934 are included in the graph. There is a striking similarity in the curves for all four years, despite the marked difference in the yearly totals.

During the epidemic year, therefore, the age-group incidence was identical with that of non-epidemic years. The slight secondary rise following the age of 20 years was almost entirely due to cases among young mothers of infant cases.

DISCUSSION

The serological findings are in accord with those investigations which have failed to demonstrate the serological unity of the scarlatinal streptococci. The variation from month to month in the proportion of cases due to the various serological types may serve to explain, in part, the discrepancies noted in previous investigations of this nature. The importance of prolonging the collection of strains from acute cases over a considerable period of time, rather than carrying out an examination of strains gathered during a limited interval, is well illustrated by the variation in the number of type 5 cases. In June, this type was responsible for 78 per cent of all cases, but in September of the same year, for only 42 per cent, i.e. the proportion had fallen by almost half. A limited examination in June only would have led, almost certainly, to the conclusion that the majority of

strains from acute scarlatina were of one serological type, whereas a consideration of the September results alone was against such a finding.

Reviewing shortly the age groups in which the cases occurred, there was a remarkable lack of differentiation as between the non-epidemic years 1931, 1932 and 1934 and the epidemic year 1933. Yet during those years there was a very marked difference in the actual number of cases, in 1931 there being a total of 564 and, in 1933, of 3461 or a six-fold increase. Thus during the epidemic year of 1933 all age groups were equally affected by the epidemic since the increase in the number of cases was proportionate in all age groups, the characteristic maximum age-group incidence between the ages of 5 and 9 years being maintained. As far as the exposed population was concerned, there appeared to be two possible explanations for this proportionate increase in case incidence. Firstly, the causal factors of the epidemic were universally at work in all age groups, or alternatively, an increase in the cases at some particular age group was attended by a secondary increase in all other age groups. In the latter event the rise in the primarily affected group would have preceded in time the secondary cases. Instead, the increase was simultaneous in all groups, pointing to the existence of some universal

contributory factor.

How far variation in the properties of the causal organism, the haemolytic streptococcus, can be identified with this factor requires further consideration. In this connection the present investigation revealed several interesting points. Firstly, there was a marked preponderance of one particular type of haemolytic streptococcus, namely 5, during the two-month period preceding the onset of the epidemic. Secondly, the proportion of type 5 cases rose with the early development of the outbreak but had reached a maximum before the epidemic was numerically at its worst. Type 5 at this latter period was still responsible for more cases than any other single type, but the increasing magnitude of the epidemic was maintained by a rise in the proportion of the other type s present and, in particular, of type 3. The outbreak of the epidemic was therefore associated with an overwhelming predominance of a single type, but there was a suggestion that it was maintained by a succession of rises in the incidence of all the types present at the outset rather than the continued multiplication of the original predominant type. No evidence was obtained regarding the particular function or property of the organism upon which this rise in the case incidence may depend. Detailed results of an investigation of the toxin production of certain of these

strains, isolated at different periods of the epidemic, have been previously reported (Green,1935). In brief, this study showed that there was no significant variation in the property of toxin production among the strains studied.

There was a distinct tendency for type 5 and type 3 strains to be found with relative frequency in the discharge examination of convalescents, from whom some other type had been isolated in the acute stage. This may have been due to an increased capacity of these particular strains to spread from patient to patient, but a more likely explanation is to be found in the fact that these same strains were in much greater concentration in all wards from the acute stage onwards.

The result of hospitalisation was to reduce the number of streptococci in the throat to none or very few in the majority of patients, and in only 5.4 per cent of discharges was there a sufficiently large number present to give rise to any doubt as to the possibility of return cases appearing as a result.

In the greater proportion of those cases in which haemolytic streptococci were recovered on discharge the type present was identical with that isolated on admission. This would appear to indicate that the scarlatinal strains exhibit serological stability to a considerable degree. In a certain number

of discharged patients, however, an additional strain was recovered which failed to agglutinate with specific sera against the most frequently encountered types. Continued growth of the original infecting strains in the secretions of persons undergoing active immunisation against those strains was thought to be a possible explanation for this inagglutinable type of organism. An attempt to restore the original type characteristics by raising the virulence by animal passage was not successful. This observation has since been confirmed by Neisser(1939) who reported that strains from convalescents tended to be less easily agglutinated and frequently seemed to have lost the type-specific agglutinogens.

SUMMARY AND CONCLUSIONS

1. Eight serological types of haemolytic streptococci were recognised in acute scarlatina during the 1933 epidemic in Edinburgh.
2. Five of these types were identical with Griffith's types 1,2,3,4 and 5, while the remainder have been named provisionally, B,C and D.
3. Type 5 was predominant during the two months preceding the epidemic and throughout the early development of the outbreak.
4. The epidemic was maintained by successive increases in the proportion of cases due to the remaining types, particularly type 3.

5. The age-group incidence in the epidemic year was identical with that of non-epidemic years.

6. 36.8 per cent of patients on discharge were found to have haemolytic streptococci in the throat but in only 5.4 per cent was a large number of organisms isolated.

7. Haemolytic streptococci isolated from discharged convalescents were in the majority of cases of the same type as the admission strain.

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SECTION B

SEROLOGICAL EXAMINATION OF HAEMOLYTIC STREPTOCOCCI FROM ACUTE RHEUMATIC AND CONTROL GROUPS

INTRODUCTION

The association between inflammation of the upper respiratory tract and the rheumatic state has long held clinical recognition. The evidence on which this has been based was supported by the frequency with which tonsilitis occurred in rheumatic patients, statistical data being supplied by the work of St. Lawrence(1920), Ingerman and Wilson(1924), Poynton(1925), Bertram(1925), and McCulloch and Irvine-Jones(1929). In these investigations the incidence of throat infections was found to vary from 22.4 per cent to 77 per cent. Additional proof of the prevalence of repeated infection was found in the condition of the fauces. Thus the St. Thomas's Hospital data in the Medical Research Council(1927) Report on rheumatism showed that the proportion of healthy throats in children of non-rheumatic families was greater than that in rheumatic families. In a similar controlled investigation Lambert(1920) found that in 1,000 consecutive cases of rheumatism the proportion of unhealthy tonsils was 25.3 per cent, as compared with

17 per cent in 250 cases of acute pneumonia. Active infection as indicated by inflammation of the throat was present in 22.4 per cent and 0 per cent of rheumatic and control groups respectively. Perhaps a more striking demonstration of the connection between the two conditions is supplied by the occurrence of outbreaks of rheumatism, mainly of recurrent attacks but sometimes primary, after epidemic tonsilitis. Many examples of this sequence are described in the literature by Raven (1923), Boas and Schwartz (1926), Hiller and Graef (1927-8), Glover (1930), Schlesinger (1930), Glover and Griffith (1931), Collis (1931), Sheldon (1931), Bradley (1932), and Coburn and Pauli (1935).

While there is thus considerable evidence for the occurrence of rheumatism after tonsilitis, the bacteriological examination of the throat flora in such cases has yielded divergent results. Although the streptococcal genus has attracted most attention, many species within the genus have been incriminated by different observers. Thus the work of Poynton and Paine (1900) stressed the importance of the alpha haemolytic or viridans streptococci. On the other hand, Small (1927) and Birkhaug (1927) separately claimed aetiological significance for gamma or indifferent streptococci. Within recent years, however, beta haemolytic strains have gained increasing recognition in the reports of Glover (1930), Coburn (1931), Glover and

Griffith(1931), Collis(1931), and Bradley(1932). Similar investigations, such as that described by Schlesinger(1930), have led to the view that no single type of organism could be recognised as responsible for all infections, but that many species were involved. It may be noted that in the majority of investigations in which beta haemolytic streptococci have appeared important the type of naso-pharyngeal infection has been of the epidemic variety in enclosed populations such as those of schools, hospitals, and training quarters. Thus the importance of Mantles observation in 1885, at the beginning of the bacteriological era, that rheumatism was a more common complication of infectious sore throat than of sore throat of a non-infectious nature, becomes manifest. Even in sporadic cases of rheumatism, such as in the series of Gibson, Thomson and Stewart(1933), beta haemolytic streptococci were isolated from the throat secretions in 43 per cent of patients, as compared with 20 per cent of controls. It is commonly recognised that haemolytic streptococci from human sources differ greatly in pathogenicity as determined by virulence tests in experimental animals; but the method is not suitable for extensive investigations, nor does the pathogenicity in man and animal always concur. Lancefield(1933), by means of a precipitin reaction, claimed that haemolytic streptococci could

be divided into groups, each of which possessed a common, group-specific carbohydrate fraction(M). Furthermore, Group A represented the important pathogens in the human subject. An important practical application of this test was the demonstration by Lancefield and Hare (1935) that the majority of strains causing puerperal infection of the uterus were Group A in character, whereas the majority of strains in the birth canal during a normal puerperium were not. As Lancefield (1935) suggested, the main source and habitat of Group A strains is probably the human naso-pharynx, but not all commensal strains in this site belong to Group A. Hare (1935) for instance, examining the nose and throat of normal human being, noted that only about one-third of haemolytic streptococci isolated were Group A, the carrier rate of this type in a normal population being estimated to be approximately 7 per cent. The relatively low proportion of commensal Group A strains in normal persons has been confirmed by Davis and Guzdar (1936), who recorded a carrier rate of 3 per cent. Again, Plummer (1935) classified all but two of 418 strains from human infections in Group A, but found that many strains from healthy subjects were not of this group. However, a much higher proportion of carriers has been recorded by Kodama (1937), who found that 68 per cent of fifty normal subjects were in this category.

Clearly this method of examination may yield information concerning the part played by haemolytic streptococcal infection of the naso-pharynx in rheumatic subjects, and this section records data obtained its application.

METHODS

Isolation of strains

On admission to hospital with rheumatic manifestations a throat swab from every patient was plated on 5 per cent horse-blood agar. Incubation was maintained at 37°C. for 24 hours under aerobic conditions. If colonies of haemolytic streptococci appeared, several were replated, and from the second plate, if a pure growth resulted, stock cultures were prepared by inoculation of a tube of cooked meat medium. After twenty-four hours' incubation at 37°C. the stock culture was stored at 0°C. Throat swabs from control subjects were similarly treated.

Precipitin reactions

Rabbit antisera against representative strains of Lancefield groups A to H were prepared by serial inoculation of formalinised cultures over a period of six to ten weeks. The carbohydrate antigen required for the test was prepared by inoculating a 5 c.cm. tube of horse-digest medium from the stock culture of the strain under investigation. After twelve hours'

incubation at 37°C. the 5 c.cm. broth culture was added to 50 c.cm. of the same medium. The heavy growth after eighteen hours' incubation was deposited by centrifugation and washed four times in sterile normal saline. After the final washing 2 c.cm. of sterile saline, containing hydrochloric acid to a concentration of N/20, was added to the deposited cells, and the mixture transferred to a water bath at 100°C. for ten minutes. After rapid cooling and centrifugation the supernatant extract was removed and neutralised by the addition of sodium hydroxide. The clear supernatant fluid after centrifugation was the antigen used in the test.

EXPERIMENTAL OBSERVATIONS

For this investigation two groups of subjects were selected, each numbering 200 and constituted as follows:

Group R.- Patients admitted to hospital with one or more symptoms of acute rheumatism, including arthritis, chorea, carditis, etc. This group included subjects with both initial and recurring attacks, which were of sporadic distribution in and around Edinburgh.

Group NR.- Patients in the same hospital wards as members of Group R, but without existing or previous manifestations of rheumatism. This group included all types of diseases, and no discrimination was made as

to the presence or absence of nasopharyngeal infection, as will be seen hereafter.

History of throat infections

At the time when a throat swab was taken every patient was questioned as to the occurrence of any symptoms of nasopharyngeal infection during the preceding six weeks. As indicated in Table I(p.39), in 156, or 78 per cent, of the rheumatic group R a history of such an infection was obtained. On admission to hospital with rheumatic manifestations the throat symptoms had entirely subsided in the majority of cases, although enlarged tonsils were often found. Turning to the control group (NR), a high figure of recent infection was also found- namely, in 93, or 46 per cent.

Incidence of haemolytic streptococci in throat secretions

From the throat swabs taken on admission from Group R, haemolytic streptococci were isolated in 116, or 58 per cent of patients. In the case of the control group (NR), fifty-nine, or 30 per cent, were found to be carriers. Considering the results in respect of recent throat infection, ninety-five, or 60 per cent of the 156 rheumatic patients with a positive history gave positive cultures of haemolytic streptococci, whereas twenty-one, or 47 per cent, of the re-

remaining forty-four with a negative history yielded positive cultures. In the control group (NR) the haemolytic streptococcal carrier rates in these patients with and without known recent streptococcal infection were 28 and 30 per cent respectively.

Serological types of strains.

Applying the precipitin test in the examination of all strains of haemolytic streptococci isolated from the two groups of patients the results detailed in Table II (p.39) were obtained. The most important of the data secured was the high proportion of Group A strains isolated from Group R patients as compared with Group NR. Whereas the difference in incidence of haemolytic streptococci- namely, 58 per cent and 30 per cent respectively- in these two groups was notable, the difference in the incidence of Group A infection was even more significant, the respective incidences being 50.5 and 12.5 per cent. Further, it was found that although the majority of persons in the control group with a history of recent infection were Group A carriers, only seven of thirty-three persons without recent symptoms were carriers. On the other hand, rheumatic subjects irrespective of subjective symptoms of infection yielded Group A strains in the majority of instances where haemolytic streptococci were isolated.

SECTION B TABLES I and II

TABLE I.—*Results of Throat Swab Examination in Rheumatic Group (R) and in Control Group (NR)*

	Rheumatic Group (R)	Non-Rheumatic Group (NR)
History of recent nasopharyngeal infection	156 (78%)	93 (46%)
Haemolytic streptococci isolated ..	95	26
No history of recent nasopharyngeal infection	44 (22%)	107 (54%)
Haemolytic streptococci isolated ..	21	33
Total number from whom haemolytic streptococci were isolated..	116 (58%)	59 (30%)

TABLE II.—*Serological Grouping of Haemolytic Streptococci Isolated from Throat Swabs of Both Groups*

Lancefield Group	Rheumatic Group (R)				Non-Rheumatic Group (NR)			
	No. of Strains from Patients with Recent Nasopharyngeal Infection	No. of Strains from Patients with no Recent Nasopharyngeal Infection	Total	Percentage of Total No. of Strains (116)	No. of Strains from Patients with Recent Nasopharyngeal Infection	No. of Strains from Patients with no Recent Nasopharyngeal Infection	Total	Percentage of Total No. of Strains (59)
A	84	17	101	87	18	7	25	42
B	2	1	3	3	2	7	9	15
C	4	2	6	5	3	13	16	27
D	0	0	0	0	0	1	1	2
E	0	0	0	0	0	0	0	0
F	0	0	0	0	0	0	0	0
G	5	1	6	5	2	4	6	11
H	0	0	0	0	1	1	2	3
Total	95	21	116	100	26	33	59	100

COMMENTARY

The number of subjects in each of the two groups examined was considered large enough to enable reasonable comparison to be made. A high incidence of sore throats in the rheumatic group- namely, 78 per cent- was not unique, but approximated to that found by St. Lawrence (1920) and Ingerman and Wilson (1924). In an unselected control group it was surprising to find the incidence of recent sore throats to be as high as 46 per cent, but for purposes of comparison in regard to the bacteriological investigations this was a fortunate circumstance. As haemolytic streptococci were recovered from 58 per cent of all rheumatic subjects on admission to hospital an indication was obtained of the extent to which this type of organism was responsible for the preceding throat infection noted in many of the series examined. It is recognised that faucial infections with this organism, as in scarlet fever, are followed by the carrier condition for indefinite periods, and it was reasonable to assume the same sequence in the present investigation. Of rheumatic patients with subjective symptoms of preceding infection 60 per cent yielded haemolytic streptococci, whereas in the symptomless the carrier rate of 44 per cent was still high. On the other hand, in the control group, despite the prevalence of apparent recent

infection, only 30 per cent yielded haemolytic streptococci, and approximately the same proportion of carriers was found in those with and without a recent history. This would suggest either that in the control group many of the throat infections were due to organisms other than haemolytic streptococci, or that the carrier condition after faucial infection was less persistent than in rheumatic subjects.

Regarding the Lancefield grouping of the isolated strains, it was interesting to find that 87 per cent of those from rheumatic subjects were of the important pathogenic Group A, whereas only 42 per cent of strains from controls were of this type. Nevertheless even in the control group the majority of carrier strains from persons with a definite history of recent infection were also members of Group A.

CONCLUSIONS

1. Of a group of 200 subjects with acute rheumatic manifestations 78 per cent gave a history of antecedent throat infections ; 46 per cent of a non-rheumatic control group gave a similar history.
2. Throat swab cultures for haemolytic streptococci were positive in 58 per cent and 30 per cent of the rheumatic and control groups respectively.
3. Of the strains of haemolytic streptococci from throat swabs of rheumatic subjects 87 per cent were

serologically classified in Lancefield Group A ; 42 per cent of strains from non-rheumatic controls were of this group. Group A strains were therefore isolated from 50.5 per cent and 12.5 per cent of the rheumatic and control groups.

4. The evidence supports the relationship between infection with haemolytic streptococci and the rheumatic state.

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SECTION C

THE SENSITIVITY OF RHEUMATIC SUBJECTS TO STREPTOCOCCAL PRODUCTS

INTRODUCTION

Ever since the association between streptococcal infection and the rheumatic state was first suggested, efforts have been made to gain further insight into the aetiology of rheumatism by the study of the intradermal reactions of patients to streptococcal products.

It is commonly recognised that the percentage of Dick-positive reactors among rheumatic subjects differs little from that in the general population. The condition does not therefore appear to be related to lack of antibody to the erythrogenic toxin. This does not exclude the possibility that other exotoxins play some part, but so far they remain unrecognised.

On the other hand, there has accumulated considerable evidence to suggestthat part of the rheumatic syndrome may be an expression of the allergic or hypersensitive state. The work of Swift, Derick and Hitchcock(1928) led to the conclusion that the hypersensitivity of the individual was more important than the specificity of the infecting organism. In this way the divergent findings of different investigators as to the causative organism could be reconciled.

Birkhaug (1929) found that a common allergenic factor was present to a relatively great extent in viridans and indifferent streptococci, and to a less extent in haemolytic streptococci. Irvine-Jones (1928) also reported on the non-specific nature of the allergic reaction to streptococcus extracts. Coburn (1931) has been able, however, to utilise this method in associating rheumatism particularly with naso-pharyngeal infection due to haemolytic streptococci. Collis (1931) has corroborated this work, but Gibson, Thomson and Stewart (1933) concluded that although positive reactions to haemolytic streptococcal endotoxin were more common in rheumatic than in control cases, skin sensitivity could not be regarded as an indication of a special reactivity necessary for the production of acute rheumatic infection. On the assumption that hypersensitivity was an important factor in the production of the rheumatic state, treatment by desensitisation has been attempted.. Collis and Sheldon (1932) employed intravenous vaccination with haemolytic streptococci with moderately successful results. Treatment had to be stopped in the presence of focal infection of the throat with haemolytic streptococci.

In Edinburgh the frequent association of naso-pharyngeal infection due to haemolytic streptococci has been reported by Green (1938, Section B). Many of

the patients examined in that investigation have now been examined for hypersensitivity to various streptococcal products.

METHODS

Preparation of heat-labile exotoxin

From a stock culture of *Streptococcus haemolyticus* in Robertson's cooked meat medium, stored at -4°C., 400 c.c. of horse-muscle digest broth were inoculated. After 24 hours' incubation at 37°C. the organisms were separated by centrifugation and the supernatant fluid passed through a Seitz E.K. filter. Fifty c.c. of the culture-filtrate were added to 150 c.c. absolute alcohol and the mixture vigorously shaken for 15 minutes at room temperature. The resulting precipitate was removed by centrifugation and dissolved in 10 c.c. normal saline and the reaction adjusted to pH 4.2 by the addition of 10N, HCl. At this point, the bulk of the endotoxin present was precipitated while the specific exotoxin remained in solution. The purification process was twice repeated. The final solution was adjusted to pH 7, and for skin-test purposes was diluted until 0.2 c.c. gave reactions in human subjects comparable to those produced by standard Dick toxin(Burroughes-Wellcome).

Preparation of heat-stable endotoxin nucleoprotein

The organisms separated by centrifugation from the cultures in the preparation of exotoxin were thrice washed in sterile saline with intermediate mechanical shaking for one hour on each occasion. To the centrifuged deposit 100 c.c. of absolute alcohol was added and the suspension was shaken for one hour and placed at 0°C. for 3 hours. The alcohol was removed by centrifuging and the process repeated, the organisms being left in alcohol for 12 hours at 0°C., after which the alcohol was again removed by centrifuging. The treated organisms were dried *in vacuo* and ground in a glass mill for 72 hours and then taken up in 50 c.c. phosphate buffer solution at pH 8. The mixture was shaken for 6 hours and then centrifuged at 3000 r.p.m. for 30 minutes. The slightly opalescent supernatant fluid was removed and carefully brought to pH 4.2 by the addition of 10N. HCl. A light flocculent deposit separated and was removed by centrifugation. This deposit, on drying *in vacuo*, was found to contain not less than 94 per cent of protein. When required for testing purposes an amount of dried powder equivalent to 20 mg. of protein was dissolved in 100 c.c. of boric acid-borate buffer solution and passed through a Berkfeld V filter. The skin test dose was 0.2 c.c. of a 1:100 dilution

of this solution and therefore contained 0.004 mg. of protein.

Similar endotoxin fractions were prepared from strains of viridans and indifferent streptococci.

Mixed haemolytic streptococcal exotoxin and endotoxin

For a special purpose, mixtures of endotoxin and exotoxin were prepared from the commonest types of Str. haemolyticus occurring in Edinburgh, Griffith's types 1,2,3,4 and 5 and three others provisionally numbered B,C and D(Green,1937). The mixed exotoxin was prepared by mixing equal parts of purified toxin and the mixed endotoxin by dissolving 5 mgm. of the purified protein fraction from each type (40 mg. protein in all) in 200 c.c. boric acid-borate buffer solution and treating as before. The skin test dose was 0.2 c.c. of a 1: 100 dilution in buffer solution and contained, as before, 0.0004 mg. of protein.

RESULTS

Skin reactions to products of autogenous strains

At the outset an attempt was made to test with preparations from autogenous strains of streptococci isolated from throat swabs of acute cases of rheumatism on admission to hospital. Only those subjects were selected from whom haemolytic, viridans and indifferent streptococci were all isolated. The pre-

parations used were the purified Dick toxin and the purified endotoxins of the haemolytic (H.S.E.), viridans (V.S.E.) and indifferent (I.S.E.) streptococci. In all, 32 cases of acute rheumatism were tested within 10 days of admission to hospital with the results shown in Table I (p.51). Only cases with multiple articular manifestations along with lesions such as endocarditis, pericarditis etc. were included in this series. Of the 32 cases, 7 gave positive reactions to Dick toxin. On the other hand 27 patients reacted positively to H.S.E., while 14 were sensitive to V.S.E. and 13 to I.S.E. Although 14 persons reacted to V.S.E., 11 of these were also sensitive to H.S.E. and only 3 reacted to V.S.E. alone. Similarly 11 of the 13 subjects reacting to I.S.E. also reacted to H.S.E. Further in those patients who reacted to all three endotoxin fractions, the reaction to H.S.E. was almost invariably the greatest.

Skin reactions to mixed products

While the susceptibility to erythrogenic exotoxin was no more than was expected in an average population and agreed with the accepted findings in regard to Dick reactions in rheumatism, the frequency of reactions to H.S.E. indicated that particular attention should be paid to this in an extended series of cases. The use of autogenous products had proved exceedingly laborious and was impracticable for a

large group. Accordingly the mixed solutions described above were used for intradermal tests on two large groups of patients. In a group R were included all cases with acute and subacute rheumatism, including erythema nodosum and chorea. The other group (N.R.) was composed of non-rheumatic patients and included all types of diseases. Nasopharyngeal infection due to Streptococcus haemolyticus had been a frequent precursor in the rheumatic group and to a rather less extent in the control group. The results obtained are detailed in Table II (p.51). There was again no significant difference in the percentage of positive reactors to exotoxin in the two groups, namely 24.8 and 20.4. Even in the choreic group, aged 5-10 years, 8 of 12 patients were Dick-positive. The response to endotoxin, however, revealed a definite difference between the two groups, as a positive reaction was present in 71.4 per cent of Group R as compared with 23.3 per cent in Group NR. Further, the percentage of positives in patients in the acute stage of illness was higher than in the subacute phase, except in the small group of choreics where only half were positive.

General reactions to endotoxin

In view of these findings it appeared to be rational to attempt to desensitise rheumatic patients. Serial subcutaneous injections of H.S.E. were given

SECTION C TABLES I and II

TABLE I.

Reactions to intradermal injection of streptococcal toxins in cases of acute rheumatism.

Number of patients.	Hæmolytic streptococcus.		*Str. viridans.*	Indifferent streptococcus.
	Exotoxin.	Endotoxin.	Endotoxin.	Endotoxin.
3	+	+	+	+
8	−	+	+	+
12	−	+	−	−
4	+	+	−	−
3	−	−	+	−
2	−	−	−	+
Total 32 . .	7+	27+	14+	13+
Average diameter of + reactions (mm.)	21	28	22	19
Average diameter of − reactions (mm.)	<5	<5	5	<5

+ = zone of erythema not less than 15 mm. in diameter.
− = zone of erythema less than 15 mm. in diameter.

TABLE II.

Reactions to intradermal injection of hæmolytic streptococcal products in cases of acute and subacute rheumatism.

Type of case.	Number tested.	Reactions to endotoxin.		Reactions to exotoxin.	
		+	−	+	−
Group R.					
Acute rheumatism . .	55	43	12	10	45
Subacute rheumatism . .	29	20	9	6	23
Erythema nodosum . .	9	6	3	2	4
Chorea	12	6	6	8	4
Number . .	105	75	30	26	76
Per cent. . .		71·4	28·6	24·8	75·2
Group N.R.					
Non-rheumatic controls—					
Number . .	103	24	79	21	82
Per cent. . .		23·3	76·7	20·4	79·6

+ and − as in table I.

and in the majority of cases provoked no response other than slight local discomfort. In a few individuals certain suggestive phenomena were encountered. The larger doses of H.S.E. induced a local reaction which closely resembled the lesion of erythema nodosum. The site of inoculation became swollen, tender and even brawny in appearance. No ulceration followed and the visible reaction, starting 6-8 hours after inoculation, reached its maximum in 24 hours and subsided within 48-72 hours. In no instance did such lesions appear elsewhere than at the site of inoculation. In five other patients generalised reactions were produced in which rise of temperature, tachycardia and a return of flitting point pains were temporarily present. The reaction passed off in 24-48 hours but the patient was still sensitive and reacted in a similar manner to further injections. One case may be briefly described:-

Mrs. H.C., aged 29 years. The only points of interest in the previous history were " growing pains" at 8-12 years and scarlet fever at 19 years. Five years before admission to hospital the patient suddenly became ill with head ache and shivering. Two days later, she felt pain in the ankle, elbow, wrist and finger joints. The knee and shoulder joints were in turn affected. Salicylates alleviated the pain and the patient was transferred to hospital. On admission

there was still slight pain in the knee, ankle and elbow joints. The heart was apparently healthy. Intradermal injection of 1 skin-test dose of H.S.E. produced a zone of erythema 30 x 30 mm. A series of injections of a preparation of H.S.E. containing 500 skin-test doses per c.c. was given subcutaneously. As seen in the chart (p.54), the initial doses of 0.1, 0.2 and 0.4 c.c. were tolerated without the slightest reaction but the fourth dose, 0.8 c.c., resulted in the appearance of a large, hot, raised, painful swelling locally and a general reaction in which the hyperpyrexia, tachycardia and return of joint pains simulated a true relapse. In view of the absence of cardiac complications treatment was continued. Four further injections induced similar reactions, the possibility that these were naturally occurring incidents being excluded by the strict time relationship between injection and response, together with the absence of reactions on cessation of treatment. A feature of this case was the delay in the occurrence of reactions until after the fourth injection when a total of 1.5 c.c. of H.S.E. had been given. The temperature, previously subnormal, began to rise 18 hours after the injection, reached a peak of 104.2°F at the 24th. hour and was again subnormal within 36 hours. Despite the increasing dosage the subsequent

SECTION C CHART 1

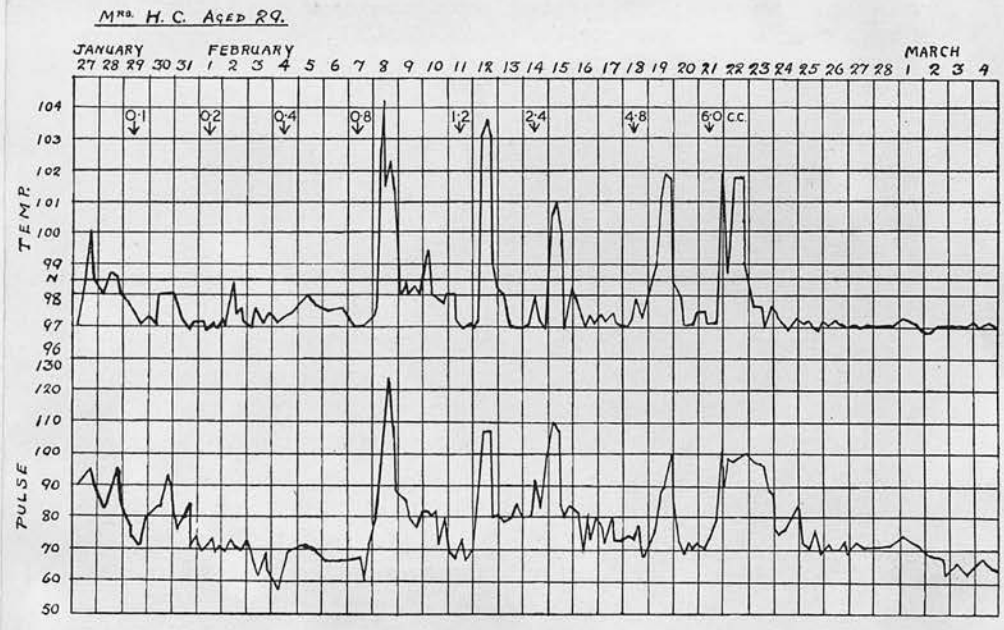

CHART.—Temperature and pulse reactions of a quiescent rheumatic subject to a series of subcutaneous injections of hæmolytic streptococcal endotoxin.

reactions were slightly less intense than the first. At no time during treatment was there evidence of either organic or functional cardiac disturbance.

DISCUSSION

The general results corroborate the findings of other workers that rheumatic subjects are, as a group, more frequently sensitive to streptococcal endotoxin than non-rheumatic controls. Although sensitivity was not specific for any one kind of streptococcus, it was demonstrated with greatest regularity by the use of the haemolytic streptococcal product. The antigenic constitution of the nucleo-protein or heat-stable endotoxin is at present unknown and it may contain one or more fractions. It is therefore impossible to state whether the frequency of reactions to H.S.E. is due to a greater content of an antigen common to all three kinds of streptococcus or to some peculiar specific fraction present in addition to the common antigen. As each of the small group of cases in which this point was investigated had yielded streptococci of all three kinds on throat swab culture, the increased sensitivity to endotoxin of Str. haemolyticus was not simply associated with the presance of this organism in the throat to the exclusion of other species. On the other hand cases of acute rheumatism were encountered in which no hypersensitivity

to H.S.E. was demonstrable by intradermal tests. The use of autogenous endotoxin in the smaller series had produced 27 or 84.4 per cent of positive reactions, whereas the mixed product in the extended series of 105 cases induced 71.4 per cent. Possibly an even higher figure would have resulted from the autogenous endotoxin had it been used throughout. The occurrence of negative reactors was to some extent corroborative of the conclusion reached by Gibson, Thomson and Stewart that skin sensitivity could not be regarded as an indication of a special reactivity necessary for the production of rheumatic infection, but it did not detract from the possibility that part of the rheumatic syndrome is due to hypersensitivity. As an analogy, cases of active tuberculosis, both in the human and bovine species, may occasionally fail to react to tuberculin despite the recognised part played by allergy in this condition.

A more striking demonstration of the sensitivity of rheumatic subjects to haemolytic streptococci was afforded by the occasional untoward results attending attempted desensitisation by subcutaneous injection of H.S.E. Localised reactions resembling erythema nodosum and generalised reactions, indistinguishable from naturally occurring relapses but for their transient nature, were thus induced. Although this cannot be regarded as a specific attribute of H.S.E.,

as similar reactions have been produced by the intravenous injection of vaccines of Str. viridans (Swift, Hitchcock and Derick, 1927-28), the delayed type of reaction was distinct from the immediate or rapid response of non-specific protein shock therapy. The fact that such reactions occurred indicates the need for extreme care in the use of vaccines or bacterial extracts in such patients.

SUMMARY

1. Of 32 cases of acute rheumatism, 27, 14 and 13 showed skin sensitivity to endotoxin of autogenous haemolytic, viridans and indifferent streptococci respectively.

2. Seventy-five per cent of a series of 105 cases of acute and subacute rheumatism gave positive skin reactions with a stock preparation of haemolytic streptococcal endotoxin as compared with 24 per cent of 105 non-rheumatic controls.

3. In a limited number of quiescent cases, local and general manifestations of the rheumatic syndrome have been induced by subcutaneous injection of haemolytic streptococcal endotoxin.

4. The evidence supports the view that haemolytic streptococcal infection is an important factor in the production of the rheumatic state.

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SECTION D

ANTISTREPTOLYSIN O TITRES OF VARIOUS CLINICAL GROUPS

INTRODUCTION

The introduction to this subject started in the description of the antigenic nature of the haemolytic filtrates of streptococcal cultures in serum-free broth by Todd (1932a) who later (1934) demonstrated the group specificity of the products, and detailed the titration of a neutralising antibody to be distinguished in further work as antistreptolysin O. Todd (1938a, 1938b, 1939) found that Group A (Lancefield) haemolytic streptococci produce two serologically distict varieties of haemolysin. On account of oxygen sensitivity one variety was designated streptolysin O, and the other streptolysin S in virtue of its extractability in serum. The response in animals to experimental infection with whole cultures of Group A streptococci was a rise in titre of the neutralising antibodies, antistreptolysin O (A.S.O.) and antistreptolysin S(A.S.S.). This later work has not rendered less important the application of antistreptolysin O

titration in the detection of haemolytic streptococcal infection.

Todd (1932 b) first reported the A.S.O. titres of normal human sera to be in the zone of 3-100 units per c.c.,and demonstrated a rise in titre of varying degree after known streptococcal infection such as scarlatina, erysipelas etc., Coburn and Pauli (1935 a) supported these observations by reporting that student nurses before exposure to haemolytic streptococcal infection had A.S.O. titres of from 33 to 100 units, with one exception of 167, the mean for the group being 83. In nurses who contracted infection, the titres were later found to range from 117 to 333 units, the mean being 200, while nurses who escaped infection remained at the originally low level. In a later communication, the same authors (1935 b) noted that the mean of A.S.O. titres in 176 individuals in good health was 71 units.

On the basis of these and similar results, the titration of A.S.O. has been applied to the study of streptococcal infection in acute rheumatism. Todd (1932 b) reported a rise in titre during the active stage of rheumatic fever, which was confirmed by Coburn and Pauli(1932). Studying the results of a wave of haemolytic streptococcal infection in a group of rheumatic children, all in the quiescent phase with carditis, Coburn and Pauli (1935 c, 1935 d) recorded that 14 of 16 individuals infected with the epidemic strain, developed acute rheumatism, the onset of

symptoms being accompanied by a coincident rise in A.S.O. titre. Seven children who escaped infection also failed to develop acute symptoms and showed no rise in titre. The titres of two patients infected with the epidemic strain remained unaltered and they also escaped acute manifestations. The same authors (1935 b) stated that the median titre developed in acute rheumatism was 500 units, and the geometric mean in their series was 490 units.

In comparing the A.S.O. response in different clinical groups of small size, Coburn (1936 a) found that in scarlatina the maximum titre was reached in 17 days, and in non-rheumatic pharyngitis within 10 days. The titres rapidly declined thereafter in both groups. In all rheumatic children who contracted streptococcal pharyngitis followed by rheumatic recrudescence there was an increase in titre. In mild cases this reached its highest limit within 19 days, but in severe cases the maximum titres were not reached until much later, e.g. 34, 36 and 80 days after the onset of infection.

Studies by Coburn and Pauli (1939), on the response in non-rheumatic scarlatina and pharyngitis in a larger series of cases, led them to extend the period within which the maximum titre was attained in the absence of complications to 28 days. An important observation was the influence of complications in delaying the maximum response beyond this period.

Further data have been presented by Todd, Coburn and Hill (1939) who observed a significant shift to higher figures in the A.S.O. titres of rheumatic subjects who contracted pharyngitis without recrudescence of rheumatism, as compared with non-rheumatic children with pharyngitis or scarlatina. An even greater response was evident in rheumatic children who relapsed after throat infection.

Similar observations over a period of three years are now described.

METHODS

Determination of antistreptolysin O titre

The method described by Todd (1934) was adopted, with the modification in rearrangement of dilutions suggested by Coburn (1936 b). Streptolysin O was prepared from the "Richards" Griffith type 3 strain of Streptococcus haemolyticus.

Assessment of clinical activity

All cases were assessed by one observer on the basis of presence or absence of joint lesions, indications of cardiac involvement, variation in temperature and pulse rate, sedimentation rate and alteration in formol-gel reaction (section L : Green, Thomson and Glazebrook, 1939).

EXPERIMENTAL OBSERVATIONS

The clinical subjects were all males, 96 per cent being in the age-group sixteen to eighteen years.

Available for study were 65 sera from 20 cases of scarlatina: 83 sera from 33 cases of haemolytic streptococcal pharyngitis: 1092 sera from 137 cases of acute or subacute rheumatism. It was not possible to obtain sera from healthy boys in the same age group, but 106 sera were taken from healthy boys in the age-group eighteen to twenty years. These youths had been in the same environment as the other groups for a minimum period of two months.

Of the various correlations which could be made with the available data, that concerning the relationship between A.S.O. titre and acute rheumatism was first examined by grouping cases showing a similar range of titre.

Range of antistreptolysin O titre in acute rheumatism

The range of antistreptolysin O titre during the active and inactive phases of 110 acute rheumatic attacks is presented in Table I(p.66). The cases were divided according as the clinical picture was of the monocyclic or polycyclic form. The results were next grouped according to the extent of the titre range i.e. the difference between the highest and lowest readings recorded throughout the particular attack. The group showing maximum variation included all cases with the titre range represented by a difference in end-point of 6 or more tubes in the neutralisation test or titration. The moderate variation group was similarly represented by a difference in

end-point of 3 to 5 tubes. Any variations in these two groups could be considered significant. The minimum variation group included all cases whose serial titrations remained at a constant level. Also included in this group were cases in which differences of only 1 to 3 tubes existed, these being considered insignificant under the conditions of the test.

Table I(p.66) shows that 87.5 per cent of the monocyclic cases were accompanied by a significant change in titre. This figure included 78.2 per cent in which titres during the active phase of rheumatism were higher than in the inactive phase, and 6.3 per cent in which the titres during inactivity were the higher. No titre below 100 units was recorded in the monocyclic cases with constant titres, 7.8 per cent being in the 100to 249 unit zone, and 4.7 per cent at 250 units or over.

In the polycyclic series, 93.5 per cent of cases showed a significant variation. In 82.6 per cent the active-phase titres were higher than the inactive. In no case were all the inactive titres higher, although significant change in titre occurred without detectable clinical alteration in 10.9 per cent. Of the three cases with fixed titres in all atages, two were in the 0-99 unit zone and one at 100-249 units.

Thus 80 per cent of all cases showed a response indicative of recent streptococcal infection, 10 per

cent failed to respond and 3.6 per cent presented a reversed effect.

Range of Antistreptolysin O titre in scarlatina and in haemolytic streptococcal pharyngitis

From each of 15 cases of scarlatina and 24 cases of haemolytic streptococcal pharyngitis, a sufficient number of titrations were available to determine the range during active infection and convalescence, the results being added to Table I(p.66) . In scarlatina, all titres during activity were higher than in convalescence, 11 of the 15 cases exhibiting a maximum variation. Of the 24 cases of pharyngitis, a maximum reaction was noted in only 7, and in 8 cases a moderate increase in titre during activity occurred. The remaining 9 cases showed no appreciable change although all the titres were over 100. As sera from several of these cases were not obtained until four to seven days after the onset on infection, it is possible, though unlikely, that a transient early reaction was missed.

For purposes of comparison, the data was next examined in the manner followed by Todd, Coburn and Hill (1939) .

Distribution of antistreptolysin O titres in various clinical groups

The combined titration results of sera from various clinical sources are presented in Table II (p. 68)

TABLE 1 -- TO SHOW RANGE OF ANTISTREPTOLYSIN O TITRE IN UNITS PER C.CM. SERUM IN SCARLATINA, HAEMOLYTIC STREPTOCOCCAL PHARYNGITIS AND IN MONOCYCLIC AND POLYCYCLIC FORMS OF ACUTE RHEUMATISM.

Range of Antistreptolysin O Titre	Acute Rheumatic Cases						Non-Rheumatic Cases	
	Clinical Type				All Cases		Scarlatina	Pharyngitis
	Monocyclic		Polycyclic					
	No.	%	No.	%	No.	%	No.	No.
Maximum Variation								
Titres higher in active phase	22	34.5	27	58,7	49	44.5	11	7
Titres lower in active phase	0	0	0	0	0	0	0	0
Titres varying in same phase	1	1.5	4	8.7	5	4.5	0	0
	23	36.0	31	67.4	54	49.0	11	7
Moderate Variation								
Titres higher in active phase	28	43.7	11	23.9	39	35.4	4	8
Titres lower in active phase	4	6.3	0	0	4.	3.6	0	0
Titres varying in same phase	1	1.5	1	2.2	2	1.8	0	0
	33	51.5	12	26.1	45	40.8	4	8
Minimum Variation								
Titre range in zone of 0-99	0	0	2	4.3	2	1.8	0	0
" " " " "100-249	5	7.8	1	2.2	6	5.5	0	7
" " " " 250 and over	3	4.7	0	0	3	2.8	0	2
	8	12.5	3	6.5	11	10.1	0	9
	64	100.0	46	100.0	110	99.9	15	24

I. Healthy subjects:

As previously indicated, the sera from healthy subjects were taken at random without regard to history of recent pharyngitis for which there were no bacteriological data. The distribution of the A.S.O. titres in this group (col.I, Table 2) was such that 82.3 per cent were less than 125 units per c.c. and only 2.8 above 250, the arithmetic mean being 79.

2. Non-rheumatic pharyngitis and scarlatina:

The combined distribution of these two groups (col.4) represented a marked shift to higher figures, 72.4 per cent being over 125 and 46 per cent above 250, the arithmetic mean being 280. The scarlatina figures (col.3) were somewhat higher than those for simple pharyngitis (col.2), the arithmetic means being 300 and 263 respectively.

3. Rheumatic Group:

The general effect of rheumatic activity was obtained by comparing the distribution of all sera collected during active and inactive periods (col. 7) and 8). The distribution of titres during quiescent periods was intermediate between that of the normal controls and the non-rheumatic pharyngitis group, but much nearer the latter. The A.S.O. titres of 66 per cent of the inactive period sera were 125 units or more, and 34.6 per cent were over 250 units. With the development of rheumatic activity, the distribution was raised much higher, 90.7 per cent being over 125

TABLE II

Table 11. DISTRIBUTION OF ANTISTREPTOLYSIN O VALUES OBSERVED IN SERA OF DIFFERENT GROUPS.

Anti-streptolysin O units per c.cm. of serum	Non-Rheumatic				Rheumatic			
	Healthy Controls	Pharyngitis	Scarlatina	Pharyngitis and Scarlatina	Pharyngitis		Active periods	Inactive periods
					No rheumatic attack	With rheumatic attack		
	Col.1	Col.2	Col.3	Col.4	Col.5	Col.6	Col.7	Col.8
	Absolute Numbers Observed							
16-82	70	19	7	26	3	7	41	73
83-124	18	11	4	15	4	2	23	62
125-249	16	19	20	39	7	9	145	125
250-399	2	14	18	32	4	18	205	85
400-624	1	12	5	17	0	10	121	40
625-832	0	3	5	8	0	11	61	7
833-999	0	0	0	0	0	0	18	1
1000 or more	0	5	6	11	0	4	79	5
Total Sera	107	83	65	148	18	61	694	398
Average	79	263	300	280	188	420	444	210
	Percentage Frequencies							
16-82	65.3	22.7	10.8	17.5	.	11.5	5.9	18.3
83-124	17.0	13.5	6.2	10.0	.	3.2	3.3	15.6
125-249	14.9	22.9	30.8	26.4	.	14.7	21.0	31.4
250-399	1.9	16.8	27.8	21.7	.	29.5	29.6	21.4
400-624	0.9	14.6	7.6	11.5	.	16.4	17.4	10.0
625-832	0	3.5	7.6	5.4	.	18.0	8.8	1.7
833-999	0	0	0	0	.	0	2.6	0.3
1000 or more	0	6.0	9.3	7.4	.	6.6	11.3	1.2
	Percentage Frequencies in Condensed Form							
16-249	97.2	59.1	47.8	55.9	.	29.4	30.2	65.3
250-624	2.8	31.4	35.4	33.2	.	45.9	47.0	31.4
625 or more	0	9.5	16.9	12.8	.	24.6	22.7	3.2

* Pre- and post-activity.

units and 69.7 per cent over 250. Whereas only 3.2 per cent of sera during inactivity were 625 units or more, the active group produced 22.7 per cent at this high level. The arithmetic means of the active and inactive groups were 444 and 210 respectively.

4. Pharyngitis in rheumatic subjects

While under observation, 20 rheumatic subjects in the quiescent state contracted haemolytic streptococcal pharyngitis. Following upon this throat infection, 16 of the 20 patients developed acute rheumatic manifestations. Seventeen attacks were followed, one subject having two such lapses, and the results are collected in Table II, col. 6 (p.68) . The distribution of the titres of 61 sera from this group was very similar to that in the active rheumatic series (col.7), as were the arithmetic means.

Four patients experienced streptococcal pharyngitis without reawakening rheumatic infection, and in all four there was no rise in A.S.O. titre. Only 18 sera were available from this group which was too small for purposes of significant comparison. It may be noted, however, that 14 of the 18 sera had titres of less than 250 units and none were above 400.

Degree of rheumatic activity

In Table III(p.70) the results are grouped according to the degree of clinical activity. Only 12 sera were obtained during the period immediately preceding activity, but all were below 400 units and the

TABLE 111 --- TO SHOW DISTRIBUTION OF ANTISTREPTOLYSIN O VALUES OBSERVED IN SERA DURING RHEUMATIC ATTACK, ACCORDING TO DEGREE OF RHEUMATIC ACTIVITY.

Antistreptolysin O units per c.cm. of serum	Rheumatic Activity				
	Preactive	Increasing	Maximum	Decreasing	Doubtful
	Absolute Numbers Observed				
16-82	4	0	12	11	14
83-124	1	1	1	13	7
125-249	1	9	31	55	50
250-399	6	15	53	102	67
400-624	0	5	37	53	26
625-832	0	4	19	25	13
833-999	0	2	7	8	1
1000 or more	0	1	31	33	14
Total Sera	12	34	181	286	181
Average	167	412	538	450	363
	Percentage Frequencies				
16-82	33.4	0	6.6	3.7	7.7
83-124	8.3	2.9	0.6	4.5	3.9
125-249	8.3	26.4	17.1	19.2	27.6
250-399	50.0	35.3	23.8	31.3	31.0
400-624	0	15.7	20.3	18.2	14.4
625-832	0	11.8	10.5	8.8	7.2
833-999	0	5.9	3.9	2.8	0.5
1000 or more	0	2.9	17.1	11.6	7.7
	100.0	99.9	99.9	100.1	100.0
	Percentage Frequencies in Condensed Form				
16-249	50.0	29.3	24.3	27.4	39.2
250-624	50.0	50.0	44.1	49.5	45.4
625 or more	0	20.6	31.5	23.2	15.4

mean of 167 was the lowest observed in any phase of the illness. During increasing activity the distribution shifted up the scale, the mean titre reaching 412 units. The peak was attained at the stage of greatest activity with a mean of 538 units. Even at this point, 6.6 per cent of specimens were below 82 units. With diminishing activity, the figures tended to return to lower values but remained at relatively high levels as compared with those in the quiescent phase (cf.Table II,p.68). The arithmetic mean in the immediate post-active phase was 363 units.

Effect of time since infection in scarlatina and pharyngitis

From cases of scarlatina, only six sera were taken in the first 6 days of illness. The titres of five were less than 125 units, and the remaining titre was 159 units (Table IV, p.72). In the second week of illness, 71.5 per cent of specimens were more than 125 units, and in the third week the highest distribution was observed, 65.3 per cent being 250 or more. The figures during the fourth and subsequent weeks showed a regression to lower values.

The distribution in simple pharyngitis approximated closely to that in scarlatina with only minor differences. Thus higher titres were observed at the commencement of illness, but in the following weeks the distribution was at a slightly lower level than in scarlatina. Nevertheless, the highest distribution

TABLE IV

TABLE IV - DISTRIBUTION OF ANTISTREPTOLYSIN O VALUES OBSERVED IN SERA FROM SCARLATINA AND HAEMOLYTIC STREPTOCOCCAL PHARYNGITIS, ACCORDING TO DURATION OF TIME SINCE INFECTION

Antistreptolysin O units per c.cm. serum	Scarlatina				Pharyngitis				Scarlatina & Pharyngitis			
	Interval in days from start of illness											
	0-6	7-13	14-21	22 or more	0-6	7-13	14-21	22 or more	0-6	7-13	14-21	22 or more
	Absolute Numbers Observed											
0-82	4	2	0	1	9	4	2	4	13	6	2	5
83-124	1	0	0	3	4	3	0	4	5	3	0	7
125-249	1	3	9	7	6	2	6	5	7	5	15	12
250-399	0	1	6	11	1	1	7	5	1	2	13	16
400-624	0	0	4	1	2	3	3	4	2	3	7	5
625-832	0	0	3	2	1	0	1	1	1	0	4	3
833-999	0	1	0	0	0	0	0	0	0	1	0	0
1000 or more	0	0	4	1	0	1	3	1	0	1	7	2
	Condensed Table											
0-124	5	2	0	4	13	7	2	8	18	9	2	12
125-249	1	3	9	7	6	2	6	5	7	5	15	12
250-624	0	1	10	12	3	4	10	9	3	5	20	21
625 or more	0	1	7	3	1	1	4	2	1	2	11	5
	Percentage Frequencies											
0-124	83.3	28.6	0	15,4	56.5	50.0	9.1	33.3	62.0	42.9	4.2	24.0
125-249	16.6	42.9	34.7	26.9	26.1	14.3	27.3	20.8	24.2	23.8	31.2	24.0
250-624	0	14.3	38.4	46.1	13.1	28.6	45.4	37.5	10.3	23.8	41.8	42.0
625 or more	0	14.3	26.9	11.6	4.3	7.0	18.2	8.3	3.4	9.5	22.9	10.0
	99.9	100.1	100.0	100.0	100.0	99.9	100.9	99.9	99.9	100.0	100.1	100.0

was again observed during the third week. This can be correlated with the finding that nine of the pharyngitis cases (Table I, p.66) started with titres over 100 but showed no increase, whereas all the scarlatina cases responded with a marked increase in titre.

DISCUSSION

The results from the clinical groups were particularly suitable for purposes of comparison in that all the subjects were males in a strictly limited age-group of sixteen to eighteen years. The controls in healthy males were in a slightly higher age-group, viz. eighteen to twenty years. The low A.S.O. titres in the healthy controls, closely followed the results of Coburn and Pauli (1935 a), the respective means being 79 and 83. Although controls in the younger age-group were not available, little difference was to be expected from the distribution in the older controls who had been in a similar environment for at least two months before examination. If anything, the readings in younger controls would have been lower, on account of reduced opportunities, in age-years, for infection to have occurred. On the other hand, it may be argued that there did exist environmental differences in the exposure of the clinical groups and healthy controls, resulting in a greater risk of infection and higher initial titres. Apart from the

absence of any evidence of such environmental differences, this criticism is countered by the low titres of the scarlatinal and pharyngitis groups in the first week of illness, and by the relatively low titres of the small number of sera from rheumatic patients prior to the onset of activity. Further, it was the experience of Coburn and Pauli(1935 a) that close contact with an infected group did not materially increase the A.S.O. titres of non-infected controls.

The distribution of titres in non-rheumatic scarlatina and pharyngitis presented, therefore, a marked and significant shift up the scale of values, but to a lower plane than that observed by Todd, Coburn and Hill(1939). The arithmetic mean was actually higher in the present series- namely, 280 as compared with 239- but this was due to the presence of fewer extreme values. The distribution approximated more closely to that of the rheumatic group with pharyngitis but no rheumatic activity in Todd's series.

The data indicated that the maximum titre in scarlatina and pharyngitis was attained most frequently in the period from fourteen to twenty-one days after infection. Experimentally, it has been observed that the A.S.O. titre falls within a few days of the cessation of the supply of antigen (sectionF). It may be deduced, therefore, that even in simple pharyngitis and in scarlatina there is prolongation of the antigenic stimulus for a considerable time after the acute

throat infection has subsided, but that this period does not extend beyond four to five weeks. Coburn and Pauli (1939) have fully demonstrated that prolongation of the increased titre in scarlatina and in pharyngitis was due to complications or continued infection.

The results confirm previous observations on the general increase in A.S.O. titre in acute rheumatism, although the degree of increase was not as great as that reported by Todd, Coburn and Hill(1939). In presenting the results, two methods were considered essential in attempting correlation with the clinical process. The tabular recording of titre values, as followed by Todd, Coburn and Hill(1939) provided a very satisfactory method for statistical comparison of different groups. Unfortunately, the method did not disclose the various responses encountered in a single, clinical entity. Thus,in a recognised streptococcal infection such as tonsilitis, some patients failed to respond,although an increase in A.S.O. titre was the general finding in this condition, irrespective of the high or low level of the initial titre. As may be expected from the known toxigenic nature of scarlatinal strains of haemolytic streptococci, six fully observed cases of this disease exhibited well-marked reactions.

In the rheumatic group, the majority of cases

were accompanied by a significant increase in titre, but absence of response and even a fall during renewed activity was occasionally demonstrated. The variation in A.S.O. response in relation to the mechanism of acute rheumatism will be considered in the next section.

CONCLUSIONS

1. The antistreptolysin O titre of 1346 sera from male adolescents, including various clinical groups and helathy subjects, have been determined.
2. The mean titre in normal controls was 79, 82.3 per cent of the sera being less than 125 units per c.c.
3. An increase in titre was invariably noted in scarlatina, the mean being raised to 300 with maximum titres occurring in the third and fourth weeks after infection.
4. Simple pharyngitis due to Streptococcus haemolyticus was usually but not invariably followed by a rise in titre, the mean being 263 units per c.c.
5. The mean titre in the active phase of acute rheumatism was 444, as compared with 210 in the inactive stage of the disease.
6. Of 110 attacks of acute rheumatism, 79.9 per cent were accompanied by a significant increase in titre, which reached maximum proportions in the majority of cases at , or just after, the height of clinical activity: in 10.1 per cent of attacks no change in titre was observed: in 3.6 per cent of attacks the titres were reduced during the active phase.

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SECTION E

OBSERVATIONS ON THE ANTISTREPTOLYSIN O TITRE IN RELATION TO THE MECHANISM OF ACUTE RHEUMATIC FEVER

INTRODUCTION

Although Todd (1932) was first to describe the increase in antistreptolysin O (A.S.O.) titre during the active stage of acute rheumatic fever, he then considered that the titre was dependent on the frequent antecedent infection of the throat by haemolytic streptococci, and not necessarily on the intensity of the rheumatic process. Coburn and Pauli (1932) reported a precipitous rise in titre which immediately preceded the appearance of rheumatic manifestations, and concluded that this constant finding was strong evidence that the rheumatic attack had been initiated by haemolytic streptococcal infection. Pursuing the question in more detail, Coburn (1936 a) stated that the maximum titre was not reached until the symptoms and sedimentation rate had begun to subside. The high titres were retained for varying periods which extended to several months in severe cases. Coburn was also of the opinion that the rise in titre was the

result of antecedent throat infection but suggested that the delay in the appearance of antibody reaction after infection differentiated the rheumatic subject from the non-rheumatic. He then observed that the more severe cases were attended by the more tardy and prolonged A.S.O. reactions. Coburn and Pauli (1939) then demonstrated that the prolongation of the immune response affected the anti-M type specific precipitins as well as antistreptolysin O production, pointing to the continued activity of the haemolytic streptococcus as a whole, and not to a specific effect on antistreptolysin O.

In a combined report, Todd, Coburn and Hill (1939) reaffirmed that the A.S.O. titre reached its zenith at the height of the rheumatic attack or immediately afterwards. Statistical evidence in general confirmation of this report was presented in the previous section. Examples of antistreptolysin O response are now examined in relation to the mechanism of rheumatic fever.

METHODS

Determination of antistreptolysin O titre

The method described by Todd (1934) was adopted with the modification in rearrangement of dilutions suggested by Coburn (1936b). Streptolysin O was prepared from the "Richards" (Griffith type 3) strain of *Str. haemolyticus.*

In the previous section, the results were

grouped according to the extent of the titre range, which was the difference between the lowest and highest readings recorded throughout the particular illness. The group showing maximum variation included all cases with the titre range represented by a difference in end-point of six or more tubes in the neutralisation test or titration. The moderate variation group was similarly represented by a difference in end-point of three to five tubes. Any variations in the maximum and moderate groups could safely be considered significant. The minimum variation group included all cases whose serial titrations remained at a constant level. Also included in this group were cases in which differences of only one or two tubes existed, these being considered insignificant under the conditions of the test.

Estimation of erythrocyte sedimentation rate and formol-gel reaction

The results illustrated in the accompanying charts were obtained by a centrifuge tube method (section L: Green, Thomson and Glazebrook 1939), correction for haematocrit volume being made according to the formula proposed by Gibson (1939).

Assessment of clinical activity

The results were assessed by one observer on the basis of presence or absence of joint pains and lesions, indications of cardiac involvement, variation in temperature and in pulse rate.

Type of acute rheumatism

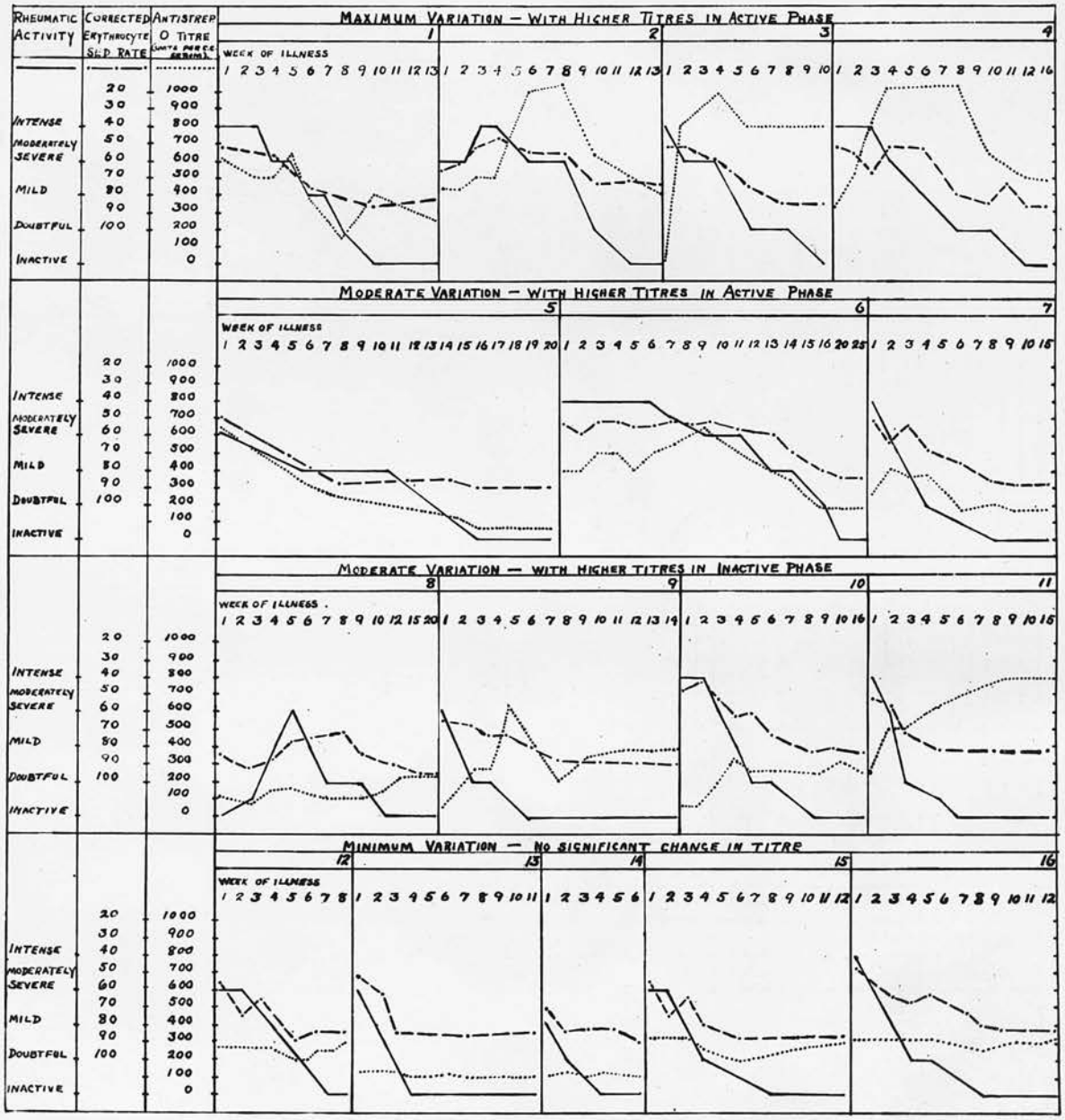
The clinical cases from which data were obtained were all males, aged sixteen to eighteen years. The cases of acute rheumatism under investigation were mainly, but not all, primary attacks in boys who had passed acertain standard of medical examination not longer than three years prior to the onset of illness. With few exceptions, this was the first recurrence, within that period, in anyone admitting a previous history of rheumatism.

Acute rheumatism manifested itself in the group with the usual symptoms of pain and stiffness in one or more joints, followed by flitting arthritis in a varying number of joints. Marked effusions were not common but transient swelling and tenderness was present in the early stages. Cardiac complications followed in a considerable proportion of cases. Although the joint symptoms were generally of a moderately severe nature, the cardiac lesions rendered the condition extremely serious.

Clinically, the cases could be arbitrarily divided into monocyclic and polycyclic types. The monocyclic group presented a single phase of activity with pyrexia and joint pains of varying degree and duration, with or without cardiac complications. In polycyclic cases two or more such attacks were separated by less intense periods in which there was some

CHART 1

CHART 1:- To show examples of Variation in Antistreptolysin O Response in Monocyclic Attacks of Acute Rheumatic Fever



indication of continuing activity.

Monocyclic cases

The statistical examination of the antistreptolysin O titres in this group indicated a significant rise in titre in 78.2 per cent of 64 cases under observation. Examples of the titration curves are illustrated in Chart I(p.82) . Cases 2,3,4,6 and 7 correspond to those described by Coburn (1936 a) as typical of the rheumatic state. Thus the maximum titre was reached after the period of greatest activity was passed, and the sedimentation rate was improving. Abnormally high titres were maintained for weeks, and in some instances for several months, but a final decrease in titre was observed after improvement in the clinical condition. In other cases, as in case I and 5 (Chart I), the titres were as high or higher at the onset of the attack as in any subsequent stage. It could not be stated that only severe cases were accompanied by a prolonged A.S.O. response. Thus case 3 was mild and of short duration without detectable cardiac complications, but the titre remained at a very high level for several months. On the other hand, all severe cases in which any rise in titre developed, presented this to a high degree. As in all other cases in this group, a final fall in titre was observed.

Cases 8,9,10 and 11 constituted the small group (6.3 per cent) in which titres during inactivity

were higher than in the active phase. In case 8, no significant shift in the titre from a resting level of 100 accompanied a severe attack, but when convalescence was well-established in the twelfth week the titre rose to 250 and was maintained at that level until the patient left observation in the twentieth week. Case 9 started with a titre of 50 at the onset of an attack of short duration. At the end of the fourth week, an increase to 625 was noted which was succeeded by a drop to 200 in early convalescence. A progressive increase followed, which finally surpassed and remained above the highest titre during activity. There was no clinical reason for this increase in titre, the patient remaining perfectly well. Cases 10 and 11 were similar to 9, save that no fall in early convalescence was noted. As a significant increase occurred during the attack in cases 9, 10 and 11, they possibly represented extreme examples of delayed reaction but no correlation of antibody response with activity of the rheumatic process could be made. The titres of cases 8, 9, 10 and 11 remained within close proximity of the final levels recorded in Chart I until the end of an observation period of six months. They were therefore placed in a separate serological category to differentiate them from the majority of cases in which a final fall in titre was noted before the end of a similar observation period, although no clinical distinction between the two groups could be made.

Cases 12, 13, 14, 15 and 16 were representative of the group (12.5 per cent) in which no significant change in titre was detected throughout the attack. Clinically these cases were also indistinguishable from the majority in which an increase had occurred. Case 15, for example, was a severe polyarthritis involving many joints before the condition finally settled. The titre was 312 at the first observation on the second day of illness, and remaindd at or about that level throughout. As in cases 12 and 14, the titres were well above the mean of 79 observed in the normal control group. The other cases illustrated were of a less severe nature, but nevertheless bore the typical character of rheumatic polyarthritis.

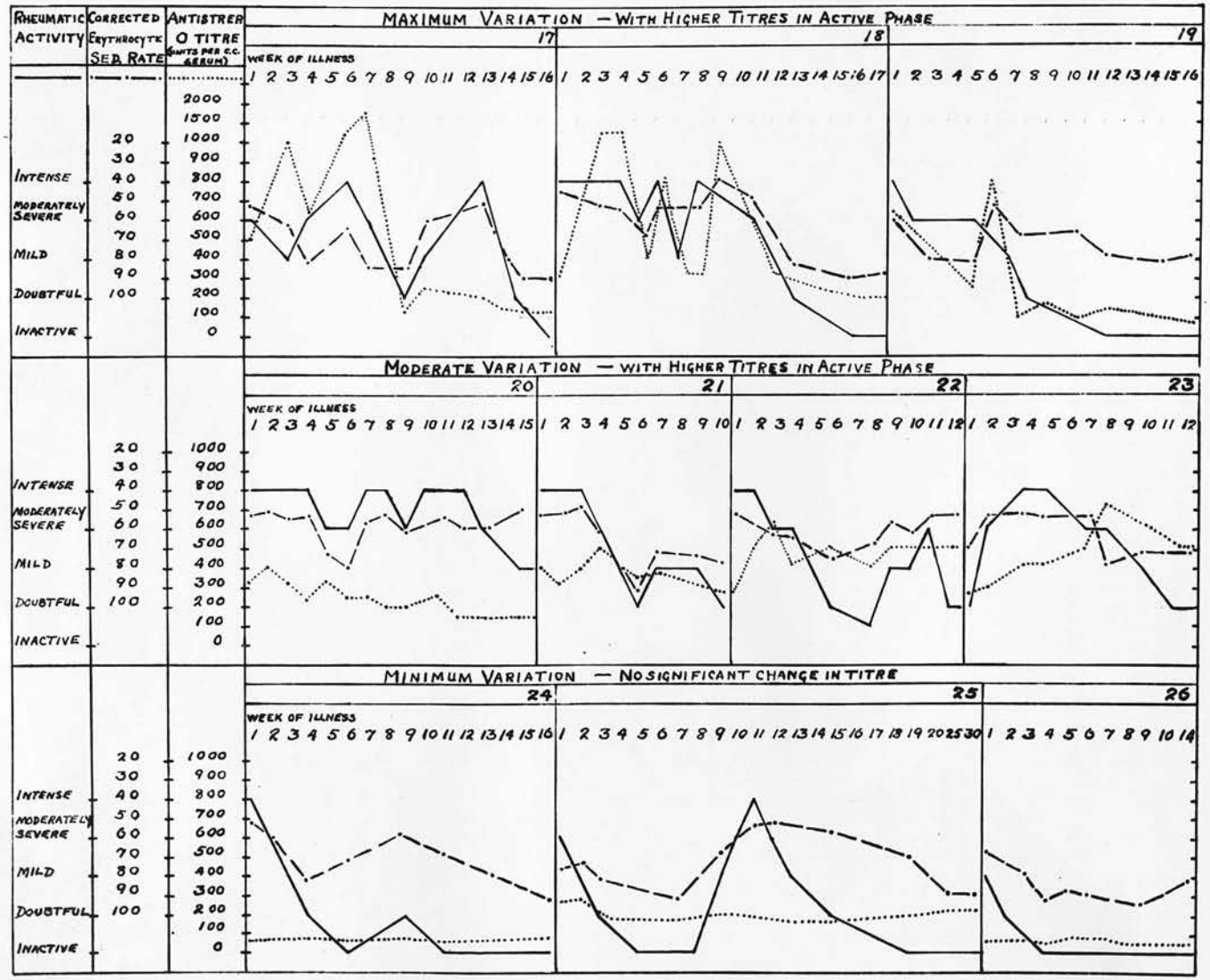
Thus the majority of cases of the monocyclic type, the least complex form of rheumatism, presented a response indicative of recent streptococcal infection, but this reaction was absent in a few cases of comparable severity.

Polycyclic cases

In 82.6 per cent of 46 polycyclic attacks, there was also a significant increase in titre during the active phase.

As shown in Chart II (p.86). these cases afforded good examples of the relationship between rise in titre and clinical crisis. In cases 17 and 18, three clinical cycles were each attended by an advance in titre, although this was not always of the same intensity.

CHART 2:- To show examples of Variation in Antistreptolysin O Response in Polycyclic Attacks of Acute Rheumatic Fever



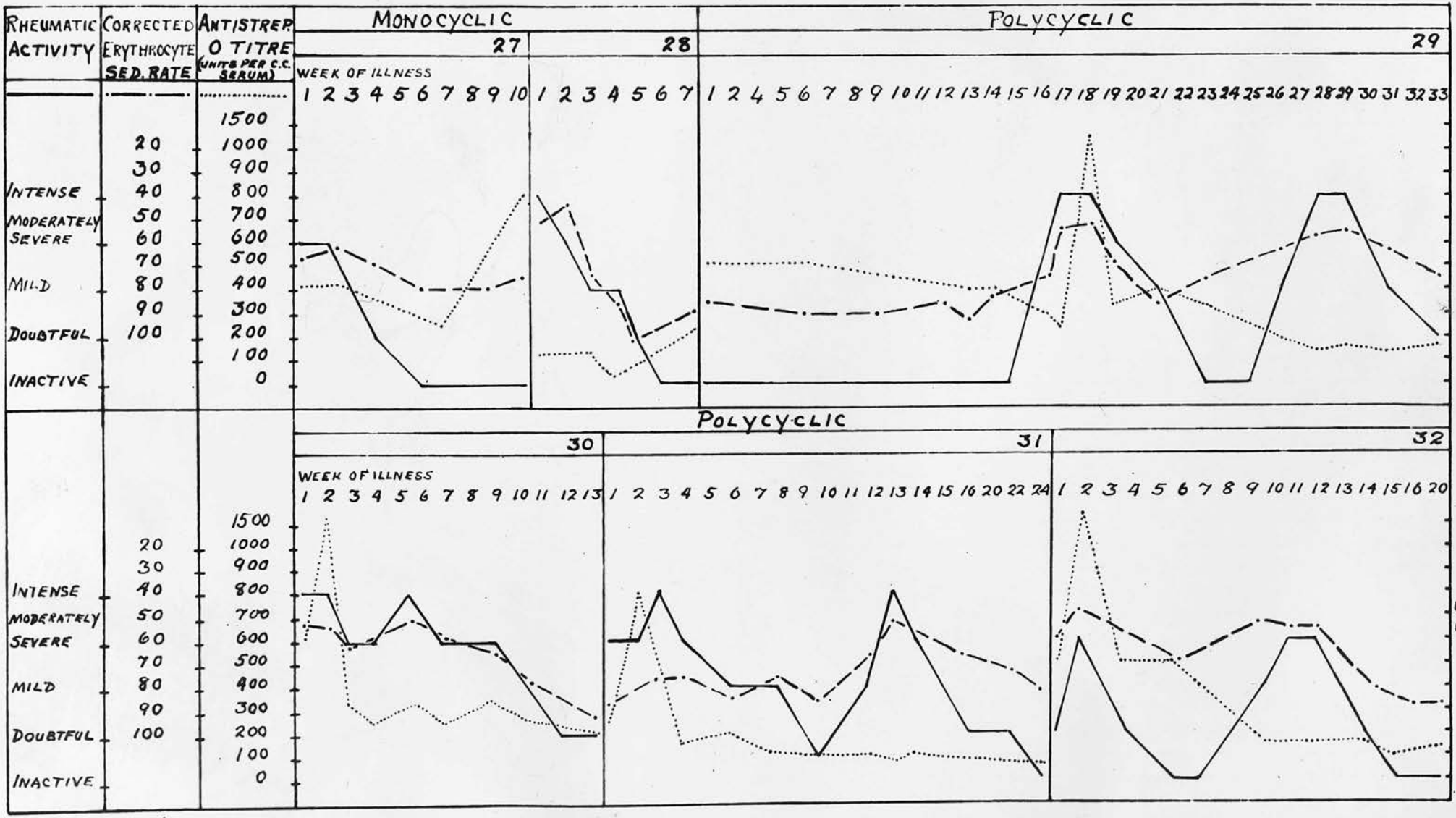
Thus the first two cycles in case 17 were followed by marked responses but the third produced a much smaller reaction. In other cases, as in 20, 21 and 22, the swing in titre between the cycles was not so evident and the titre curve remained at a high level throughout the active phase of illness.

The small group (6.5 per cent) of polycyclic cases in which there was no significant change in titre was formed by cases 24, 25 and 26. Of these, the first two were clinically severe, and although case 26 was of milder character, the titre varied no more than in the period charted for twenty-six weeks of observation.

Variation in titre without alteration in clinical state

On account of their clinical reaction, those cases showing marked variation in titre during the same clinical phase were separately illustrated in Chart III (p.88). The two monocyclic cases, 27 and 28, both had titres in early convalescence lower than those obtaining in the active phase, followed by a rise in late convalescence. The polycyclic series supplied four cases in this category which were observed for longer periods. The response of each of the four cases was similar in that the first cycle of the attack was accompanied by a sharp rise, followed by a rapid fall. One case, 30, showed an insignificant response to subsequent cycles, but the remaining

CHART 3:- To show examples of Variation in Antistreptolysin O Titre during Active and Quiescent Phases of Monocyclic and Polycyclic Attacks of Acute Rheumatic Fever.



three cases produced no such rise, although the later clinical cycles were as intense as that at the onset of illness.

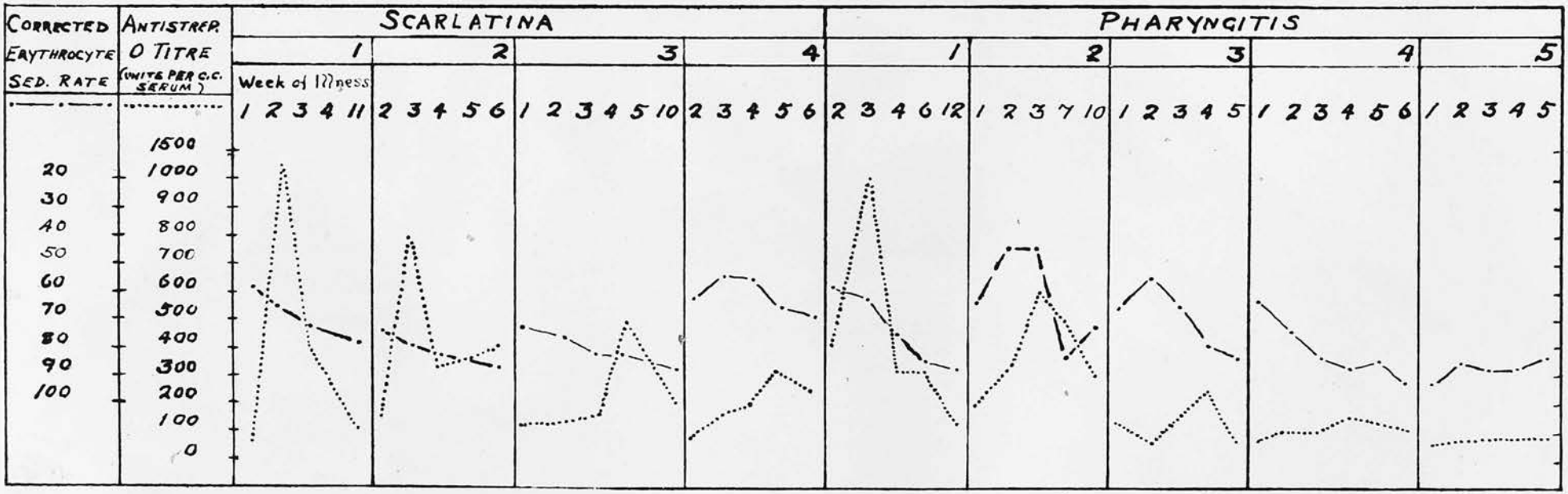
Antistreptolysin O response in scarlatina and in haemolytic streptococcal pharyngitis

The statistical examination (section D) had shown that an increase in titre was invariably noted in the cases of non-rheumatic scarlatina studied, the mean being raised to 300 with maximum titres occurring in the third and fourth weeks after infection. In simple pharyngitis due to Streptococcus haemolyticus, there was usually, but not always, a similar response and the mean titre was raised to 263.

Selected cases are illustrated in Chart 4 (p.90) Of the scarlatina group, cases 1 and 2 were typical examples of the intense but rapid reaction during the second and third weeks. Cases 3 and 4 exemplied the occurrence of maximum titres in the fourth and fifth weeks. In pharyngitis, there was also a considerable variation in response from the high, rapid peak in case 1, to the slow,weak response in case 4. The absence of any change in titre, noted in nine of the twenty-four cases of pharyngitis examined, is illustrated in case 5.

There was considerable individual variation, therefore, in this non-rheumatic group after streptococcal infection. The absence of a reaction in a minority of cases indicated that a negative result

CHART 4:- To show examples of Antistreptolysin O Curves in Scarlatina and in Haemolytic Streptococcal Pharyngitis (Non-Rheumatic)



could not be interpreted as certain evidence of the non-occurrence of recent streptococcal infection.

Haemolytic streptococcal pharyngitis in rheumatic subjects

While under observation, twenty rheumatic subjects in the quiescent state contracted pharyngitis. Four attacks were followed by uninterrupted convalescence, and in all four cases there was no increase in A.S.O. titre. Fourteen of the eighteen sera from this group had titres under 250. One case was observed through two attacks of pharyngitis, of which only the second was followed by mild rheumatic activity. The first throat infection which failed to stimulate rheumatic infection also failed to produce any increase in titre, but the second attack of pharyngitis was accompanied by a moderate rise in titre which persisted during the subsequent period of clinical activity. This finding appeared to support the observation by Coburn (1936 b) that in the absence of increase in titre after throat infection, rheumatic activity does not follow.

Following upon throat infection, sixteen of the twenty patients developed rheumatic activity, one having two such lapses. Although eleven of the attacks were accompanied by a rise in titre, no increase was evident in the remaining six. One of the latter was especially important in that it occurred in a patient in whom a previous attack of rheumatism had been accompanied by a marked increase in titre.

The failure in response was therefore not attributable to an individual peculiarity. It became evident that the absence of A.S.O. reaction did not invariably mean non-appearance of rheumatic activity.

Further exceptions were sought by noting the instances of renewed activity in rheumatic subjects who had no evidence of antecedent pharyngitis.

Reactivation of rheumatism without pharyngitis

While under observation, ten subjects in whom rheumatic activity had been quiescent for varying periods, developed acute manifestations without clinical evidence of pharyngitis. There were no subjective complaints, and no abnormality of the throat was detected in routine examination. In seven of these cases, haemolytic streptococci were isolated, before the onset of rheumatic symptoms, in the first positive throat swab afer a series of negative swabs. In six of these seven cases, the attack was followed by a significant rise in A.S.O. titre. In the remaining three cases, haemolytic streptococci were not isolated from the throat despite repeated examinations. In two of these cases, there was also an increase in titre at the time af the rheumatic incident. In the group of ten cases, rheumatic activity was accompanied by a rise of titre in eight subjects, although there was no clinical evidence of pharyngitis before or at the onset of the attack.

DISCUSSION

The evidence provided in this section, indicated the marked variation in the antistreptolysin O response of non-rheumatic subjects after infection of the throat by haemolytic streptococci. This suggested that the reaction depended, in part, on the degree of immunity enjoyed by the host. Thus, in a small group in whom the antitoxic immunity was low and in whom the infection became manifest as scarlatina, a significant increase in titre always followed. In the absence of complications, the titre declined by the fourth or fifth week. On the other hand, those individuals who had developed partial immunity and who suffered from simple tonsilitis as a result of infection, did not always respond by an increase in titre. If antistreptolysin O production was stimulated in the latter group, then this also ceased after the 4th. week. The actual proportion of non-rheumatic cases whose titres were not influenced by pharyngitis or tonsilitis was nine cases in a total of twenty-four.

The above variations must be remembered when the A.S.O. response of rheumatic subjects is considered. The shift to higher values in rheumatism was confirmed, as was the maintenance of high titres for much longer periods than in the non-rheumatic controls. Therefore, it was surprising to find that six of the seventeen acute attacks in known rheumatic subjects,

although preceded by pharyngitis of haemolytic streptococcal origin, were not accompanied by any significant increase in titre. This proportion, it may be noted, was roughly that of the non-rheumatic group whose titres were also unaffected by pharyngitis. Reactivation of rheumatism, therefore, could not be invariably linked with further increase in A.S.O. titre following repeated throat infection. However, it should be remembered that the distribution of A.S.O. titres in inactive rheumatism was much higher than in normal controls, and, therefore, the above conclusion did not exclude the possibility of further streptococcal activity, which was masked by the high titres already reached. Thus, in section F(p.101), it is shown that in animals with relatively high A.S.O. titres, the further injection of even large amounts of streptolysin O may have little or no effect on the titre. When the variation in non-rheumatic subjects was taken into account, the evidence in support of haemolytic streptococcal infection in the majority of the rheumatic group was considerably strengthened. In the absence of direct proof of continued streptococcal infection, Coburn (1936) has suggested that this evidence indicated an altered response on the part of the rheumatic subject to antecedent infection. One difficulty in the acceptance of this theory of mechanism has been stressed by noting that typical attacks recur in known rheumatic subjects without further increase

in titre.

Although direct proof of continued infection has not been obtained, this possibility is suggested in section G (p.133) which describes the recovery of haemolytic streptococci from heart-valve cultures made at post-mortem in cases of acute rheumatism. The source of the organisms in such material could not be definite ascertained, but the strain in each case was of the same serological type as that isolated in life from the throat of the patient, which appeared to exclude an extraneous origin. Heart-blood cultures were all negative for haemolytic streptococci, although terminal infection of the blood could not be absolutely excluded as a possible explanation for the presence of the organisms in heart-valve cultures.

While the importance of haemolytic streptococcal infection has been emphasised, there still remains for consideration the minority of cases of acute rheumatism in which no increase in A.S.O. titre was observed. Either these cases were similar in nature to non-rheumatic cases which failed to react to infection by demonstrable antibody production, or else such infection had no part in inducing their rheumatic state. With the evidence at present available it is impossible to exclude either alternative, but concentration of investigation on cases in this exceptional group may yield important information in regard to aetiology.

SUMMARY

The importance of haemolytic streptococcal infection in the production of acute rheumatism has been discussed by reference to the antistreptolysin O response.

Attention has been drawn to various types of reaction which may be encountered in rheumatic and non-rheumatic groups.

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SECTION F

EFFECT OF PRONTOSIL ON THE ANTISTREPTOLYSIN O TITRES OF RABBITS DURING IMMUNISATION

INTRODUCTION

The mode of action of prontosil and allied drugs has been the subject of intensive research, but there are few records of the effect of such therapy on antibody response, upon which the ultimate success of the treatment depends. Although much remains obscure, the general conclusion has now been reached that drugs of this group act mainly by producing bacteriostasis, thereby permitting the natural defences to come into full operation. Without adequate experimental evidence, the suggestion has frequently been made that actual stimulation of these defences by the drugs or their derivatives may also be a factor.

So far as haemolytic streptococcal infection is concerned, Colebrook, Buttle and O'Meara (1936) demonstrated that prontosil itself was inactive, but on administration to animals and man, the bactericidal power of the blood for small numbers of organisms was increased. This action was considered to be due to

the reduction of the inactive prontosil to p-amino-benzene-sulphonamide which had feeble bactericidal powers. Confirmation of these findings has been obtained, but the existence of some additional mechanism has been suggested to explain the effective therapeutic action of the drugs. Exploring the possibility of specific tissue stimulation by prontosil, Gay and Clark (1937) , working on experimental streptococcal infection in rabbits, could find no evidence that the cell reaction which finally accounted for the disposal of the organisms was other than local, and concluded that the action of the drug in stopping infection was by bacteriostasis. Levaditi and Vaisman (1935) recorded the *in vitro* inhibition of streptococcal haemolysin by Prontosil I (4-sulphamido-2-4-diamino-azobenzol) and concluded that certain sulphamide compounds exerted an antitoxic action. Osgood (1938) also suggested that the drugs of this group inactivated haemolysin and perhaps other toxic products as well. Gross, Cooper and Lewis (1938) found that Prontosil II produced a more marked inhibition of streptococcal haemolysin *in vitro* than did sulphonamide which had only a slight action. They also observed that the administration of sulphonamide *in vivo*, and its addition to serum, did not enhance the inhibitory effect of rabbit serum on streptococcal haemolysin. On the other hand, Huntington (1938) concluded that the slight delay in

haemolysin production induced by sulphonamide in broth cultures was possibly due to modification of the growth curve. Sulphonamide was without effect upon the formation of erythrogenic toxin *in vitro*, and it was unable to inactivate small amounts of the toxin when used in a concentration equal to, or greater than, that induced in body fluids therapeutically.

Any approach likely to assist the elucidation of the problem may be of importance and the following observations in regard to the antistreptolysin O response of rabbits were recorded. This antibody can be accurately titrated, and its elaboration as part of the natural response to haemolytic streptococcal infection has been fully demonstrated by Todd (1932).

METHODS

Source of streptolysin O

The method described by Todd (1935) for the production of streptolysin O was followed, the strain of haemolytic streptococcus being "Richards" (Lancefield, Group A-Griffith, type 3). The same batch of streptolysin was used throughout, the titre being 8.4 units per c.c.

Antistreptolysin O A.S.O. titration

The method described by Coburn and Pauli (1935) was used.

Prontosil therapy

The drug chosen for investigation was Prontosil, II, the disodium salt of

4-sulphamido-phenyl-2-azo-7-acetylamino-1-hydroxynaphtalene-3-6-disulphonic acid. Each injection of 2c.c. of 5 per cent solution was given on the left side, half into the subcutaneous tissues and half into the musculature.

Implantation of agar foci

10 c.c. of an eighteen-hour broth culture of the "Richards" strain of Str. haemolyticus was centrifuged, and the deposited organisms suspended in 4 c.c. melted agar at 40°C. Immediately, two cubic centimetres of the suspension were injected subcutaneously into the right side. The doughy mass of the inoculum persisted for several days and the site later became indurated. The regional glands were distinctly palpable for varying periods, but no fluctuation was detected and the overlying skin was not involved.

EXPERIMENTAL OBSERVATIONS

Response to streptolysin O as culture-filtrate

In the first experiment, the effect of prontosil therapy on the antistreptolysin response to serial injections of streptolysin O was studied.

Two rabbits, no.12 and no.13 (body-weight, 3010 gm. and 3420 gm. respectively) were selected and an indication of the probable response to streptolysin O was obtained. The rabbits were each given the same preliminary course of intravenous injections of

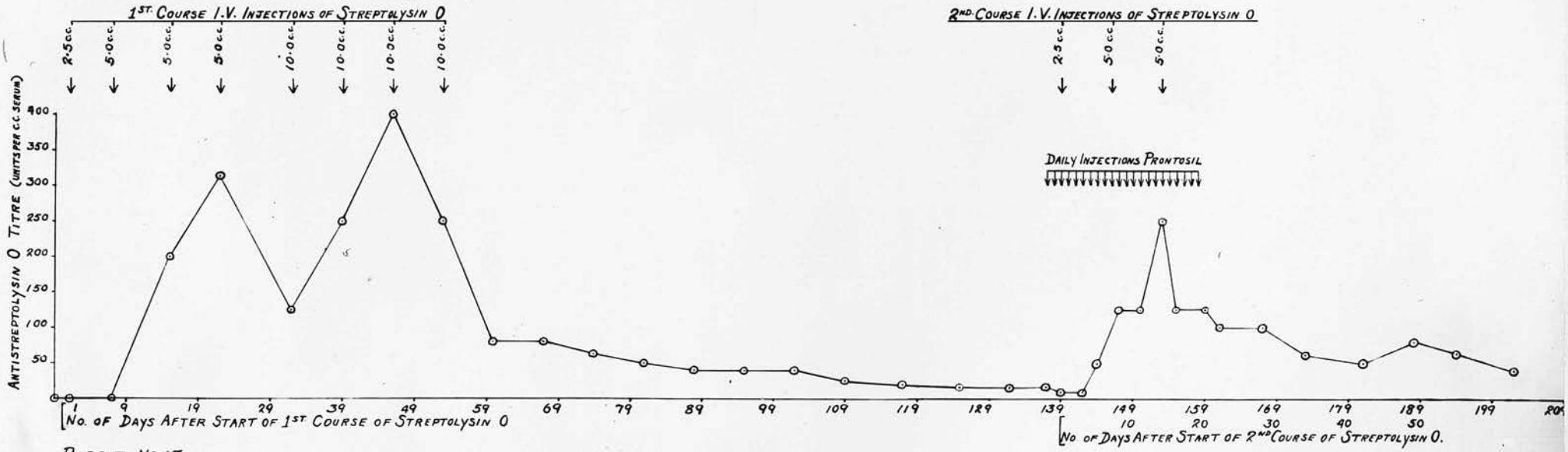
streptolysin O as shown in Chart 1 (p.102), venous blood being withdrawn for antistreptolysin O (A.S.O.) titration immediately before each injection. At the beginning of the experiment, the A.S.O. titres of both animals were <1 unit per c.c. On the fifteenth day the titre of rabbit no.12 had risen to 200 whereas no. 13 was still <1. By the twenty-second day no.13 had also responded, the titre being 125. The subsequent curves were almost identical in shape, but that of no.12 was on a higher level throughout the course of injection. Peculiar similarities in the curves were noted. Thus a fall in titre after the fourth injection on the twenty-second day occurred in both animals. The titres reached their maximum levels towards the end of the course, but a sharp depression followed the seventh and eighth injections in no.12 and after the eighth injection in no.13. After the initial drop on cessation of injections, the titres gradually fell. No.12 reached a basal titre of 16 units on the 125th. day, while the titre of no.13 fluctuated between 6 and 10 from the 109th. day.

As compared with no.13, the response in no.12 was more rapid, maintained at a higher level and reached a steady reating titre in approximately the same time.

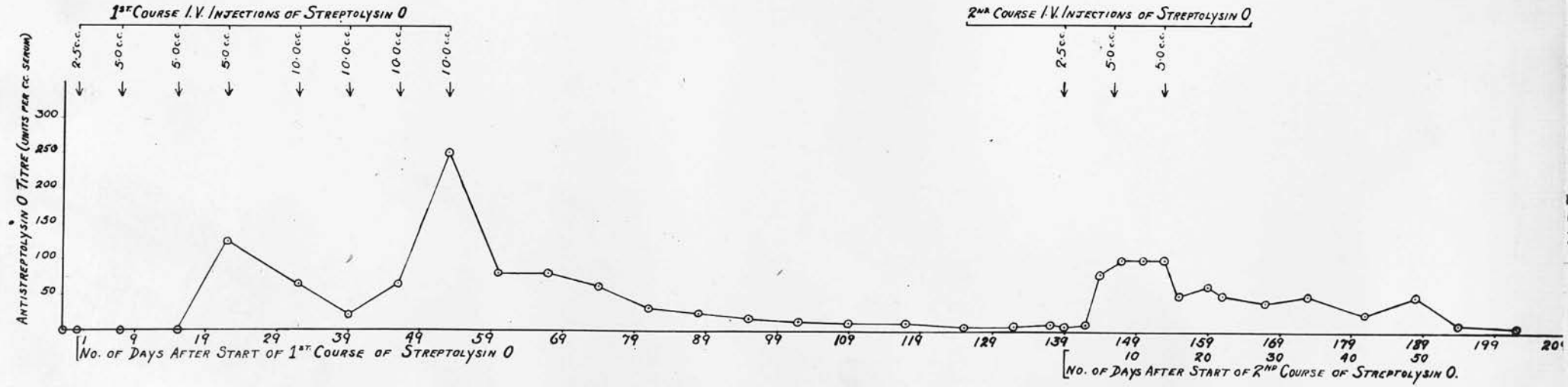
When the titres had remained steady for some three weeks, the second portion of the experiment was commenced, namely, the effect of prontosil therapy on

CHART 1:- To show the effect of Prontosil Therapy on the Antistreptolysin O response of rabbits following the injection of Streptolysin O.

Rabbit No. 12.



Rabbit No. 13



the response to a second series of streptolysin injections. Only the first three injections of the first course were repeated. On the grounds that any possible effect on response would be most manifest in the animal which had shown the greater primary reaction, no.12 was used for the test and no.13 retained as a control. Rabbit no.12 was given intravenous injections of 2 c.c. Prontosil daily from the 137th. to the 158th. day-i.e. 44 c.c. were given in a period of twenty-two days. Rabbit no.13 did not receive Prontosil. The A.S.O. titrations were performed at shorter intervals in order to detect the earliest effect. In both animals the titres rose on the sixth day after the second series of injections were commenced. The titre curves were similar save that rabbit no.12 was again at a higher level, and reached a definite peak of 250 units, whereas the curve of no. 13 was flattened at the maximum level of 100 units.

There was no indication in the preceding experiment that the streptolysin response was affected by the administration of prontosil. Nor was there any evidence of the <u>in vivo</u> production, under prontosil therapy alone, of any substance capable of neutralising streptolysin O. It will be noted that the titre of no.12 showed a slight decrease rather than increase after 48 hours therapy (139th. day).

The fact that the A.S.O. titres were <1 unit at the commencement of the experiment indicated that

streptolysin O was a completely new antigen presented to the animals. This was confirmed by the long, latent period of nine days or more before the primary response was obtained. The latent period preceding the rise in titre in the second part of the experiment was shortened to 4-6 days, this reduction being characteristic of a secondary response.

Implantation of agar foci of Str. haemolyticus

In the second experiment, the effect of prontosil therapy on antistreptolysin O response after experimental infection by haemolytic streptococci was studied.

Four rabbits, no.14, 15, 16 and 17 were selected as being of approximately equal weight. A preliminary A.S.O. titration in all gave a result of <1 unit per c.c. serum. In the first part of the experiment, no.14 and no.15 were started on daily injections of 2 c.c. Prontosil II, which were continued for twenty-three days-i.e. the total volume per animal was 46 c.c. of 5 per cent solution. After 72 hours therapy, a focus of haemolytic streptococci "Richards" strain was implanted in the right flank of both animals. The control rabbits, no.16 and no.17, received no prontosil but were given identical streptococcal foci. The streptococcal injections were repeated twenty-four hours later in all animals to ensure that infection took place.

No.16 appeared ill on the third day after in-

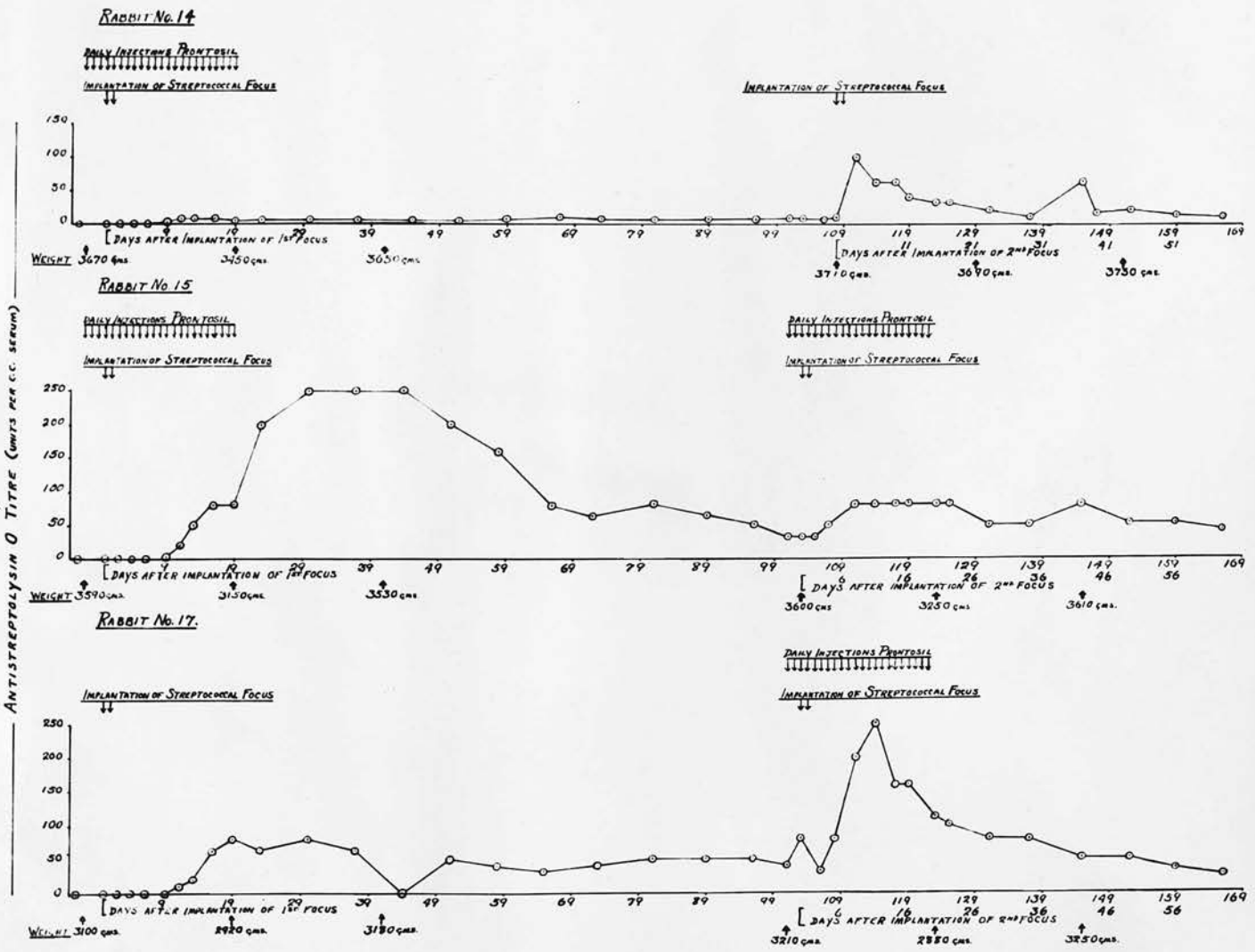
and was found dead the following morning. The post-mortem appearances and bacteriological findings indicated that haemolytic streptococcal septicaemia was the cause of death.

No.17 was in poor condition from the fourth to the eleventh day, but recovered. The A.S.O. corve is illustrated in Chart 2(p.106). The presence of this antibody was first detected on the eleventh day when the titre was 10 units. The titre reached a maximum of 80 units in nineteen days and did not fall below 40 during the first 101 days, save for an exceptional result on the 44th. day when no trace of antibody was detectable. This was the only occasion on which complete absence of antibody was observed in any animal in which it had once appeared. No explanation was forthcoming, repeated tests giving the same result.

No.14 did not appear to be in good condition from the fourth to the seventh days, but it quickly recovered, yielding the curve shown in Chart 2(p.106). The titre remained <1 unit per c.c. until the ninth day, when it rose to 1 unit. A maximum titre of 8 units per c.c. was reached in 11 days, and fluctuation between 6 and 8 units continued for over 100 days, with an exceptional reading of 10 units on the 67th. day.

No.15 lost 440 gm. body-weight from the start of the experiment to the end of the course of prontosil injections. The A.S.O. titre remained at zero until the ninth day when it was found to be 3 units per c.c.

CHART 2:- To show the effect of Prontosil therapy on the Antistreptolysin O response of rabbits following the implantation of foci of Streptococcus Haemolyticus.



The titre climbed rapidly to a maximum of 250 units on the 29th. day and maintained this level for two to three weeks. First rapidly, but later more slowly, the titre fell to 32 units on the 101st. day.

The virulence of the "Richards" strain was not tested, but it was an effective pathogen as evidenced by the fate of rabbit no.16, and the untoward effect on the other animals, particularly no.17. As indicated on Chart 2, no.14 and no.15 had lost considerable weight-220 and 440 grams respectively- when the prontosil injections were terminated after twenty-three days. This loss was largely made good in a further twenty-three days and an impression was gained that part of this loss was due to the therapy.

So far as the titration curves were concerned, the control no.17 retained a relatively high titre for a long period, considering that the maximum titre was only 80 units. The curves of the test animals, no.14 and no.15, differed very considerably and illustrated the variation in individual response following identical treatment and dosage. Had selection from a larger number of tests been possible, it would have been difficult to produce greater extremes in reaction. A feature common to both was the series of zero readings while under prontosil therapy at the start of the experiment. This confirmed the previous observation that the exhibition of prontosil did not result in the <u>in vivo</u> production of any substance capable of neutralising

streptolysin O. On the other hand, prontosil did not delay the formation of antistreptolysin O which was present at an earlier stage in treated animals than in the non-treated control. Nor did prontosil therapy appear to limit the production of the antibody since the response in one treated animal greatly exceeded the control.

In the second part of the experiment, the agar foci implanted in all three animals were identical with those of the first part. Prontosil was exhibited in different animals for the following reasons. In order to explore the possibility that the continuously low A.S.O. titre had been the result of inhibitory action by the drug, prontosil was withheld from no.14. The hypothesis of inhibition was also supported by the sharp rise in titre of no.15 on the twenty-third day, when prontosil, was stopped. In no.15, therefore, it was considered advisable to see if the result could be repeated and prontosil was given as before. The control in the first experiment, no.17, was this time given a course of the drug.

Chart 2 indicates that three days after the implantation of the focus, no.14 reached the relative-high titre of 100, which fell to 10 on the twenty-ninth day. There next appeared an unexplained rise on the 37th. day to 63 units, which dropped to 16 units on the thirty-ninth day. The contrast in the

first and second responses of this animal suggested that prontosil may have had an inhibitory action on antibody production.

Chart 2 also shows that, as before, 48 hours prontosil therapy did not alter the titre of no.15 (103rd. day). Five days after the implantation of the streptococcal focus, a rise to 50 units was first noted and on the eighth day 80 units was reached. This level was maintained until the twenty-eighth day when 50 units was recorded. Apart from a reading of 80 units on the 42nd. day, the titre subsequently remained steady at 50 units. In that the latent period before the secondary response was comparably shortened in no.14 and no.15, the use of prontosil in the latter did not delay antibody production. Otherwise the secondary response in no.15 was much less intense than its primary effort, and indicated that quantitative variations alone must be cautiously considered before acceptance as significant evidence of therapeutic interference. Nevertheless, the response was as great as the first reaction in no.17 and the second reaction in no.14, in both of these prontosil being omitted.

In no.17, the titre rose from 50 units to 80 after two days prontosil therapy. Under the conditions of the test, this rise was significant but no explanation could be found. The increase was not

maintained under continued therapy, the titre being 32 units after five days treatment, which indicated that the drug was not the likely cause of the transitory increase. Five days after the implantation of the agar focus, the secondary response was manifested in a rise of titre to 80 units, which reached a peak of 250 units on the eleventh day. The titre then fell gradually to 50 units on the forty-second day.

Despite prontosil therapy, the usual reduction in latent interval before the appearance of the response was noted, and a greater response obtained as compared with the original course. This combined result was considered positive evidence of the absence of any interference with antistreptolysin O production as a consequence of the exhibition of prontosil.

It may be noted that the test rabbits, no.15 and 17, lost 350 gm. and 330 gm. respectively- i.e. 9.7 per cent and 10.3 per cent of body-weight- in the second part of experiment 2. At the same time the non-treated control, no.14, lost only 220 grm.-i.e. 5.9 per cent of body-weight- as a result of infection alone. It may be significant that the greatest loss in weight was associated in both parts of the experiment with the use of prontosil.

DISCUSSION

Despite individual variation in response, the results indicated little, if any, interference with

antistreptolysin O production in experimental animals as a result of the injection of prontosil. It is to be expected that similar results would be found if other antibody reactions were investigated.

Apart from the academic interest of the subject, the problem is of practical importance in view of the universal application of prontosil therapy. The use of the drug is often followed by periods in which a focus of infection persists. It is important, therefore, to ascertain if there is any check to the development of the natural defence mechanism as a result of treatment with prontosil. So far as dosage was concerned, the daily injection in the experimental work described above approximated to 0.03 gm. Prontosil II per kgm. body-weight, the total amount injected over a period of twenty-two to twenty-three days being 0.66 to 0.69 gm. Neglecting species differences, the equivalent amounts for a 70 kgm. man were a daily dose of 2 gm. and a total of 64-66 gm. Larger doses are regularly used in the early days of treatment to secure a maximum effect rapidly, but there was no necessity for this procedure in the experimental work in which it was possible to institute therapy before infection occurred. Experimentally, the drug was used at least 48 hours before infection, so that any possible interference with antibody production was favoured as compared with natural infection in man, in whom the antigenic stimulus is applied, in most

cases, before therapy is commenced. A reasonable assumption is that prontosil has no appreciable antagonistic effect on antibody production in human infections. The second experiment resembled more closely the conditions under which the drug is applied in human medicine. This type of experiment, however, introduced additional variable factors, such as the amount of haemolysin liberated in animals receiving the same dose of streptococcal culture. This rendered the interpretation of results much more difficult than in the first experiment, in which an exact amount of antigen was injected. It was probable that some such variation in rabbit no.14, which accounted for the suggestion of an inhibitory effect on the part of prontosil therapy. This was the only example of the kind encountered in the investigation.

The observations may throw some light on the disappointing results of prontosil in the treatment of acute rheumatism reported by Swift, Moen and Hirst (1938). If, as suggested by Coburn (1936), the delayed and precipitous rise in A.S.O. titre after haemolytic streptococcal infection of the naso-pharynx is an essential part of the rheumatic process, then prontosil will neither prevent nor reduce the antibody response. On the other hand, the prophylactic use of prontosil in preventing throat infections and thereby eliminating the most common stimulus for increase in A.S.O. titre should be of benefit.

Encouraging results for this method of prophylaxis have already been reported by Coburn and Moore (1940).

A much more remote question upon which the touches is that of immunisation against haemolytic streptococcal infection. If it is possible so to develop control of haemolytic streptococcal infection by means of prontosil that all risk of infection is eliminated or reduced to low limits, then it may be possible to introduce a system of modified infection for improving passive and active immunisation against certain of the diseases of which this organism is the causative agent.

CONCLUSIONS

1. The effect of prontosil therapy on the production of antistreptolysin O in rabbits has been investigated.
2. No evidence of inhibition or stimulation of antibody response was obtained.
3. The serum of non-immune rabbits under prontosil treatment had no neutralising action on streptolysin O, nor were the antihaemolysin O titres of immune rabbits increased by the treatment.
4. The results are discussed in relation to the use of prontosil therapy in rheumatic fever.

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SECTION G

RHEUMATIC CARDITIS: POST-MORTEM INVESTIGATION OF NINE CONSECUTIVE CASES

INTRODUCTION

Westphal, Wassermann and Malkoff (1899) isolated streptococci from the blood, brain and heart valves of a girl dead as a result of acute endocarditis, and thereby initiated countless attempts to define the exact relationship between streptococcal infection and rheumatism. Independently and almost simultaneously Poynton and Paine (1900) recorded their first observations, in which they came to the conclusion that streptococcal infection was a cause of acute rheumatism. Among those workers who have reported confirmation of these findings may be mentioned Walker and Ryffel (1903), Beattie and Yates (1912), Lyall (1912), Swift and Kinsella (1917), and Clawson (1925). Probably an even greater number of investigators have recorded complete failure to recover organisms from any site in rheumatic subjects. Even in the successful series streptococci were recovered from the blood during life or after death, and only occasionally from the joint or cardiac lesions. These facts have given rise to the general

conclusion that the cultural findings did not support the simple explanation that rheumatism was the direct result of invasion of the joint and cardiac tissues by streptococci, but did not exclude the possibility that some association of more complex nature existed. Agonal and post-mortem bacterial invasion of the bloodstream is of frequent occurrence, as shown by Wright (1925), and must be carefully considered in relation to post-mortem cultural investigations. There was no uniformity as regards the type of streptococcus isolated by successful investigators, and this again has thrown doubt on the exact significance of their findings. In most instances, however, the organism has been of the alpha-haemolytic variety, or, according to Birkhaug (1927), Small (1927) and Kriedler (1928), the organisms may be indifferent in type. At the present time death in the acute or subacute phase of rheumatic infection is uncommon, but the series of nine consecutive cases described below were observed in Edinburgh during the space of twelve months.

METHODS

Collection of specimens

The bacteriological investigations were made during the course of the routine post-mortem examination the shortest interval between death and autopsy being fifteen hours and the longest thirty-six hours. After

opening the thorax any free fluid present in the pleural sacs was collected by means of a sterile syringe. The anterior surface of the pericardium was then seared while the heart was still *in situ*, and any free fluid in the pericardial sac was collected by means of a syringe plunged through the seared surface. The parietal pericardium was then partially reflected aseptically, and one or more pieces of any exudate present on both parietal and visceral surfaces were taken. The surface of the heart was then seared, and from 2 to 5 c.c. of heart blood were taken for culture, distributed as 1 c.c. volumes in 10 c.c. digest broth. The heart was then removed from the body, opened aseptically, and portions of any valves showing recent vegetations were cut into two or more fragments, each of which was cultured separately. No attempt was made to wash the valves free of traces of adherent blood. For controls, portions of valves without vegetations were taken from the same hearts.

Culture media

Horse digest broth: the medium described by O'Meara (1934) was used throughout in the primary culture of all specimens. In the case of fluids-e.g., pericardial fluid- 1c.c. of the specimen was added to 10 c.c. digest broth. Tissue specimens were cut into small fragments, and each cultured in a separate tube of broth.

Blood-agar plates: After twenty-four hours' incubation at 37°C. the digest broth cultures were plated on 5 per cent horse-blood agar and incubated aerobically.

Serological typing

The slide agglutination technique described by Griffith (1934) was used in the identification of strains of haemolytic streptococci isolated by the above methods.

RESULTS

Nine consecutive cases of primary or recurrent rheumatic carditis were examined by the above methods, and twenty-two non-rheumatic subjects were similarly treated as controls.

Rheumatic series

Case 1. - C.C., female, aged fifty-three years.

Clinical diagnosis.- Acute rheumatic endocarditis.

Clinical features.- Admitted to hospital in fourth attack of acute rheumatism with obvious signs of established endocarditis. She developed acute congestive failure after the subsidence of point pains. Haemolytic streptococci, type 27, were isolated from the throat ten days before death.

Post-mortem findings.- The pericardial sac was completely obliterated by fibrinous adhesions, no free fluid being present. The heart showed generalised

dilatation, with marked hypertrophy of the left ventricle and auricle.

Tricuspid valve: Areas of old fibrinous thickening, and several small, recent vegetations of rheumatic type.

Mitral valve: Gross stenosis due to old fibrous thickening of chordae tendineae, with several recent vegetations on auricular surface.

Aortic valve: Fibrous lesions, resulting from partial adhesion of cusps but no recent endocarditis.

Pulmonary valve:- Healthy.

There were no vegetations of the ulcerative type nor those associated with subacute bacterial endocardial endocarditis. The lungs, liver and spleen presented the appearances produced by venous congestion and no infarcts were detected.

Cultural results:

Heart blood..... Tubes 1 and 2. B.coli,

Tricuspid valve..... Tube 1. Haemolytic streptococci type 27, and B.coli.

Tube 2. B. coli.

Tube 3. No growth.

Mitral valve..... Tube 1. Haemolytic, streptococci, type 27.

Tube 2. Haemolytic streptococci, type 27, and B.coli,

Aortic valve..Tubes 1,2 and 3. No growth.

Pulmonary valve..Tube 1. B. coli.

" 2. No growth.

Pericardial exudate.. Tube 1. Haemolytic streptococci, type 27.

Tube 2, " " type 27.

Microscopic examination.- Sections of mitral valve, stained by haematoxylin and eosin, showed fibrous scarring with persistent vascularisation. There was superficial necrosis and new formation of vegetations, but little or no cellular reaction. Sections of myocardium showed a few small areas of fibrosis, but no Aschoff bodies. Sections of mitral valve and myocardium, stained Gram, showed no organisms.

Case 2.- G.B., male, aged twenty-two years.

Clinical diagnosis.- Rheumatic carditis, mitral stenosis and incompetence.

Clinical features.- Rheumatic fever had occurred at the age of eight years. Two years before present attack patient had been treated for auricular fibrillation. He was admitted to hospital with mitral stenosis and incompetence, and, on improvement, was transferred to a convalescent home. He continued to progress favourably until he "caught a cold" and had then to be readmitted to hospital. At this time a throat swab yielded a heavy growth of haemolytic streptococci, type 14. He then developed the typical rheumatic syndrome with severe pains in both wrists, ankles and

knees. Pericarditis supervened, and the additional stress on an already failing heart ended in death.

Post-mortem findings .- The peritoneal cavity contained more than a pint of clear serous fluid. The pericardial sac was grossly distended, containing a quart of fluid. Both parietal and visceral layers of the pericardium were covered with fresh fibrinous exudate. Posteriorly the sac was obliterated by adhesions between the two layers. All chambers of the heart were dilated, particularly the left auricle and ventricle.

Tricuspid valve: apparently healthy.

Mitral valve: both cusps slightly thickened, and the chordae greatly shortened. There was a complete line of vegetations along the margin of the valve.

Pulmonary valve: one small area of fibrosis.

Aortic valve: old-standing fibrosis, but no recent lesions.

Cultural Results:

Heart blood...Tube 1. No growth.
" 2. " " .

Pericardial fluid...Tube 1. No growth.
" 2. " " .

Pericardial exudate...Tube 1. Haemolytic streptococci, type 14.
Tube 2. " "

Tricuspid valve...Tube 1. No growth.
" 2. " "

Mitral valve...Tube 1. Haemolytic streptococci, type14, and B.coli.

Mitral valve contd/

Tube 2. B.coli.

Aortic valve...Tube 1. B.coli.

" 2. No growth.

Case 3.- D.F., female, aged thirty-three years.

Clinical diagnosis.- Acute rheumatic fever.

Clinical features.- Ten days after recovering from an attack of tonsilitis, which lasted for one week, acute polyarthritis of the limbs appeared. This continued for five weeks, when acute endocarditis was manifest. After admission to hospital haemolytic streptococci, type 18, were isolated from the throat. The patient became increasingly breathless, and died after a period of respiratory distress.

Post-mortem findings.- The pericardial sac contained about 2 c.c. of fluid, but the two layers were gummed together by fresh fibrinous exudate, and tore apart to leave the shaggy surfaces typical of an acute rheumatic pericarditis. The pleural sacs contained considerable quantities of serous effusion.

Tricuspid valve: no macroscopic lesions.

Mitral valve: typical minute vegetations along the line of closure, and a slight degree of older thickening of the posterior cusp.

Aortic valve: complete ring of minute fresh vegetations

Pulmonary valve: no macroscopic lesions.

The right upper lobe of the lung showed a patch of

confluent broncho-pneumonia which was apparently terminal.

Cultural results

Heart blood...Tubes 1 and 2. B.coli.

Mitral valve...Tube 1. Haemolytic streptococci, type 18, and B. coli.

Tube 2. Haemolytic streptococci, type 18 and B.coli.

Aortic valve...Tubes 1 and 2. Haemolytic streptococci, type 18,and B. coli.

Pulmonary valve...Tubes 1 and 2. B.coli.

Pericardial exudate...Tubes 1 and 2. Haemolytic streptococci, type 18.

Pericardial fluid...Tubes 1 and 2. No growth.

Case 4.- E. S., female, aged 14 years.

Clinical diagnosis,- Rheumatic endocarditis, terminal? meningitis.

Clinical features.- This girl had acute rheumatism at the ages, eleven and thirteen yeays. After the second attack mitral stenosis and incompetence appeared. In a convalescent home patient continued to have frequent pains in her legs and arms. Septic tonsils were removed, but the increasing dyspnoea and oedema of the legs resulted in readmission to hospital. On the second night she complained of headaches, and there was slight neck rigidity. She died at 10.30 a.m. the following morning.

Cultural results

Heart blood...Tubes 1 and 2. No growth.

Tricuspid valve...Tube 1. Haemolytic streptococci, type6.

" 2. Nomgrowth.

Mitral valve...Tube 1. Haemolytic streptococci, type 6 and B.coli.

Tube 2. Haemolytic streptococci, type6.

Aortic valve...Tubel. Haemolytic streptococci, type 6.

" 2. " " "

Pulmonary valve...Tubes 1 and 2. No growth.

Microscopic examination

Sections of mitral and aortic valves showed typical rheumatic, non-ulcerative vegetations with little cellular response. No organisms were seen in sections stained by Gram's method.

Case 5.-T.T., male, aged 4 years.

Clinical diagnosis.- Acute polyarthritis and endocarditis.

Clinical features.- Within six weeks of scarlatina the patient, who had appeared to have completely recovered, suddenly became ill with pyrexia and swelling of several joints, which were painful and extremely tender. Endocarditis supervened with a fatal result.

Post-mortem findings.- The pleural cavity contained 5 c.c. serous fluid. The pericardial sac was dry, the walls being adherent with recently formed exudate. The left ventricle was considerably dilated.

Tricuspid valve: a number of minute vegetations were present.

Mitral valve: a large number of recent vegetations bordered the free edge.

Aortic and pulmonary valves: apparently healthy.

The lower lobe of the left lung showed a pale infarct of some standing, being separated from neighbouring tissue by well-formed fibrous tissue. The right middle lobe was the seat of a chronic inflammatory process, with diffuse interstitial fibrosis, atrophy of alveoli, partial or complete obliteration of some bronchi and enlargement of others.

Cultural results

A post-mortem throat swab revealed Streptococcus viridans, pneumococci and Staphylococcus albus.

Heart blood...Tubes 1 and 2. No growth.

Tricuspid valve...Tubes 1 and 2. No growth.

Mitral valve...Tubes 1 and 2. Streptococcus viridans in pure culture.

Pulmonary valve... Tubes 1 and 2. Streptococcus viridans in pure culture.

Pericardial exudate...Tubes 1 and 2. No growth on culture.

Peritoneal fluid...Tubes 1 and 2. No growth on culture.

Microscopic examination

Sections of the left ventricle showed rheumatic nodules at a late stage. No organisms were

seen in sections stained by Gram's method.

Case 6.- N.P., male, aged 6 years.

Clinical diagnosis Acute rheumatic fever.

Clinical features This patient was admitted to hospital with a historynof polyarthritis of only one weeks' duration. Acute endocarditis followed, and death ocurred within three weeks of the onset of symptoms.

Post-mortem findings There was a large serous effusion on both sides of the thoracic cavity. A typical organisming pericarditis was present, with only a small volume of free fluid.

Tricuspid valve: apparently healthy.

Mitral valve: minute rheumatic vegetations, with slight fibrous thickening, indicating that the lesions were probably of longer duration than the history indicated.

Aortic valve: as in the case of the mitral valve.

Pulmonary valve: apparently healthy.

Cultural results

In my absence Dr.A.R. Macgregor, Pathologist to the Royal Hospital for Sick Children, Edinburgh, made the primary cultures.

Pericardial fluid:..Haemolytic streptococci, type 2, and Streptococcus viridans.

Mitral valve...Haemolytic streptococci, type 2, and Streptococcus viridans.

Microscopic examination

Sections of aortic, mitral and tricuspid valves showed recent rheumatic vegetations, and many Aschoff nodules throughout the heart muscle. No organisms were seen in Gram-stained films.

Case 8.- W.P., female, aged nine years.

Clinical diagnosis Rheumatic endocarditis and chorea.

Clinical features The initial symptoms in this case were those of chorea. After slight trauma of the knee following a fall from a car, patient returned to school but was sent home because of sickness and" raving". She commenced to kick her right foot against objects, and violent shrugging movements of the right shoulder followed. Later the left side of the body became involved. Acute endocarditis then developed, and at this stage haemolytic streptococci, type 1, were isolated from the throat. Death occurred within three weeks of the onset of endocarditis.

Post-mortem findings The serous sacs were all apparently healthy.

Tricuspid valve: no lesions were detected.

Mitral valve: numerous minute lesions were present along the line of closure.

Aortic valve: a few vegetations along the ventricular aspect.

Pulmonary valve: apparently healthy.

Cultural results

Heart blood...Tubes 1 and 2. No growth.

Tricuspid valve...Tubes 1 and 2. No growth.

Mitral valve...Tubes 1 and 2. Haemolytic streptococci, type 1.

Aortic valve...Tubes 1 and 2. No growth.

Pulmonary valve...Tubes 1 and 2. No growth.

Microscopic examination

Sections of the left and right sides of the heart were thoroughly examined but no Aschoff bodies were seen. No specific or focal changes in the brain were found other than hyperaemia and ganglion cell chromatolysis. No organisms were seen in Gram-stained sections of heart valve and muscle.

Case 9.- E.S., female, aged nine years.

Clinical diagnosis This patient was admitted to hospital with a history of pyrexia and flitting pains in the joints. On admission to hospital an active heart lesion was already present. She was later sent to a convalescent home with mitral and aortic incompetence. An attack of tonsilitis preceded a relapse, and she was readmitted to hospital with severe praecordial pain, but no evidence of endocarditis. No haemolytic streptococci were recovered from the throat. Signs of consolidation of the left lower lobe appeared and a terminal broncho-pneumonia hastened death.

Post-mortem findings The serous sacs all contained an excess of clear fluid, but there was no pericarditis. The heart was much enlarged being both dilated and hypertrophied.

Tricuspid valve: apparently healthy.

Mitral valve: almost completely encircled by recent small vegetations.

Aortic valve: several recent vegetations present, and also some older thickening of the cusps.

Pulmonary valve: apparently healthy.

Cultural results

Heart blood...Tubes 1 and 2. No growth.

Tricuspid valve...Tubes 1 and 2. No growth.

Mitral valve...Tube 1. Haemolytic streptococci, type 1.

" 2. No growth.

Aortic valve...Tubes 1 and 2. No growth.

Microscopical examination

Numerous Aschoff bodies were seen in the interlobular septa of the myocardium, but no organisms were found in Gram-stained sections.

Summarising the results, Table I(p.130) shows that from eight of this series of nine cases of rheumatic carditis haemolytic streptococci were recovered from the heart valves with vegetations and from the pericardial lesions. This organism was present in pure culture in seven valves. In six others coliform

TABLE I.—RESULTS OF POST-MORTEM BACTERIOLOGICAL EXAMINATION ON NINE CONSECUTIVE CASES OF RHEUMATIC CARDITIS

Case.	*Clinical Diagnosis.*	*Pathological State and Result of Culture.*							*Notes.*
		Tube.	*Heart Blood.*	*Tricuspid Valve.*	*Mitral Valve.*	*Aortic Valve.*	*Pulmonary Valve.*	*Pericardium.*	
1	Acute rheumatic endocarditis	1	*B. coli*	+ Hæmolytic streptococci, type 27	+ Hæmolytic streptococci, type 27	− No growth	− *B. coli*	+ Hæmolytic streptococci, type 27	Hæmolytic streptococci, type 27, isolated from throat during life.
		2	*B. coli*	*B. coli*	Hæmolytic streptococci, type 27, and *B. coli*	No growth	No growth	Hæmolytic streptococci, type 27	
		3		No growth	*B. coli*	No growth			
2	Mitral stenosis and incompetence	1	No growth	− No growth	+ Hæmolytic streptococci, type 14, and *B. coli*	− *B. coli*	−	+ Hæmolytic streptococci, type 14	Hæmolytic streptococci, type 14, isolated from throat during life.
		2	No growth	No growth	*B. coli*	No growth		Hæmolytic streptococci, type 14	
		3			No growth				
3	Acute rheumatic fever	1	*B. coli*	− No growth	+ Hæmolytic streptococci, type 18, and *B. coli*	+ Hæmolytic streptococci, type 18, and *B. coli*	− *B. coli*	+ Hæmolytic streptococci, type 18	Hæmolytic streptococci, type 18, isolated from throat during life.
		2	*B. coli*	No growth	Hæmolytic streptococci, type 18, and *B. coli*	Hæmolytic streptococci, type 18, and *B. coli*	*B. coli*	Hæmolytic streptococci, type 18	
		3	No growth						
4	Rheumatic endocarditis	1	No growth	+ Hæmolytic streptococci, type 6	+ Hæmolytic streptococci, type 6, and *B. coli*	+ Hæmolytic streptococci, type 6	− No growth	+ No growth	
		2	No growth	No growth	Hæmolytic streptococci, type 6	Hæmolytic streptococci, type 6	No growth	No growth	
5	Acute polyarthritis and endocarditis	1	No growth	+ No growth	+ *Streptococcus viridans*	−	− *Streptococcus viridans*	+ No growth	Pneumococci, *Streptococcus viridans* and *Staphylococcus albus*, isolated on culture from throat at post-mortem.
		2	No growth	No growth	*Streptococcus viridans*		*Streptococcus viridans*	No growth	
6	Acute rheumatic fever				+ Hæmolytic streptococci, type 2, and *Streptococcus viridans*	+	−	+	
7	Acute rheumatic fever	1	No growth	+ Hæmolytic streptococci, type 4	+ Hæmolytic streptococci, type 4	+ Hæmolytic streptococci, type 4	− No growth	−	Hæmolytic streptococci, type 4, isolated from throat during life.
		2	No growth	Hæmolytic streptococci, type 4	Hæmolytic streptococci, type 4	No growth	No growth		
8	Rheumatic endocarditis	1	No growth	− No growth	+ Hæmolytic streptococci, type 1	+ No growth	− No growth	−	Hæmolytic streptococci, type 1, isolated from throat during life.
		2	No growth	No growth	Hæmolytic streptococci, type 1	No growth	No growth		
9	Subacute rheumatism	1	No growth	− No growth	+ Hæmolytic streptococci, type 1	+ No growth	−	−	
		2	No growth	No growth	No growth	No growth			

+ = macroscopic lesions of recent origin present. − = no macroscopic lesions of recent origin present.

organisms were also present, and in one case Streptococcus viridans and Streptococcus haemolyticus were both obtained. No growth was obtained from three of the remaining five valves with vegetations, one yielded Streptococcus viridans and one was not cultured. As controls, twelve valves without macroscopic lesions from these same hearts were cultured, and in no instance were haemolytic streptococci recovered. Coliform organisms were obtained in three valve cultures and Streptococcus viridans in one, the remaining eight being sterile. The heart blood was sterile in six cases of the rheumatic series, coliform organisms were present in two, and in one instance this control was omitted.

Non-rheumatic series

The results obtained in the twenty-two non-rheumatic controls are summarised in Table II (p.132). Coliform organisms and haemolytic streptococci were both recovered from the heart valves of a case of post-operative peritonitis, while in one case of broncho-pneumonia haemolytic streptococci were obtained in pure culture from the heart blood and the heart valves. In both cases the pericardial cultures remained sterile, and all the heart-valve cultures yielded growths of haemolytic streptococci. As the valve inocula were not washed free of blood, the

SECTION G TABLE II

TABLE II.—RESULTS OF POST-MORTEM BACTERIOLOGICAL EXAMINATION OF TWENTY-TWO NON-RHEUMATIC CONTROL CASES.

Number of Cases.	*Result of Culture.*		
	Heart Blood.	*Heart Valves.*	*Pericardium.*
15	Sterile	Sterile	Sterile.
3	Coliform organisms	Sterile	Sterile.
2	Coliform organisms	Coliform organisms	Sterile.
1	Coliform organisms and hæmolytic streptococci	Coliform organisms and hæmolytic streptococci	Sterile.
1	Hæmolytic streptococci	Hæmolytic streptococci	Sterile.

presence of haemolytic streptococci was attributed to traces of infected blood on the valve surfaces.

DISCUSSION

The distibution of haemolytic streptococci in cultures from rheumatic and non-rheumatic hearts in the above series was striking. Unless a phenomenal chance distribution of haemolytic streptococci in the heart valves of the various cases were postulated, the results indicated that agonal or post-mortem blood invasion did not explain the appearance of haemolytic streptococci in only valves with macroscopic lesions. Coliform organisms were also isolated under the same conditions, but were found in the heart blood and in cultures from both healthy and diseased valves. Careful examination of serial sections of vegetations excluded the possibility that the positive findings were the result of subacute bacterial endocarditis. The pathological manifestations were entirely those of rheumatic endocarditis, and no organisms were found in sections stained by the routine Gram's method. Any possibility that the presence of haemolytic streptococci was the result of contamination from some source other than the tissues of the bodies under examination was completely excluded by the fact that in five cases the streptococcus recovered from the cardiac lesion, in the individual case, was of the same serological type as the strain isolated from the patient's throat

before death. Further, in those cases where haemolytic streptococci were isolated from more than one valve the strains were serologically identical. Indirect evidence has already been reported as to the part played by haemolytic streptococci in acute rheumatic infections (Green, 1938 a b, sections B and C). The observations presented in this section greatly strengthen this association, and strongly support the view that these organisms bear a causal relationship to the lesions of the disease. Although the number of cases in this series is too limited to be conclusive, they are recorded to encourage further work on the same lines.

SUMMARY AND CONCLUSIONS

1. The pericardial and valvular lesions in nine cases of acute rheumatic endocarditis were examined bacteriologically.
2. Haemolytic streptococci were cultivated from valves with microscopic lesions in eight cases, and Streptococcus viridans in one case.
3. Haemolytic streptococci could not be cultivated from valves without macroscopic lesions, nor from heart blood, in the same cases.
4. In five cases haemolytic streptococci were recovered from the throat during life, and in each case the strain was serologically identical with that isolated

from the cardiac lesions.

5. Haemolytic streptococci were cultivated from both heart blood and heart valves in two of twenty-two non-rheumatic controls.

Addendum

After the publication of the results embodied in this section, corroboration was furnished by Collis (1939) and later by Thomson and Innes (1940).

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SECTION H

RESULTS OF ANIMAL INOCULATION WITH PATHOLOGICAL MATERIAL FROM ACUTE RHEUMATISM

INTRODUCTION

Only cases 1 and 3 of the preceding section yielded pericardial fluid at post-mortem in amounts sufficient for animal inoculation, some of which proceeded to develop haemolytic streptococcal infection.

In view of the results of valve-culture previously described, it was important to compare the appearances in the experimental animals with those in the human tissues.

METHODS

Two rabbits were inoculated intravenously with 5 c.c. pericardial fluid from each case. As soon as the animals died or were sacrificed, the tissues were fixed in formol-saline and stained by various methods.

As reported in the previous section, the results obtained in the microscopic examination of human cardiac tissue by the use of the routine method

of Gram staining (Jensen modification) were entirely negative. The appearances reported in this section were obtained by extending the period of treatment with methyl-violet to six hours, the remaining stages being as before. Alternative stains were used as indicated in the figure descriptions.

Results of animal inoculation compared with direct culture

Case I : The broth culture of pericardial fluid was unfortunately omitted in this case, but the pericardial exudate yielded a profuse growth of Streptococcus haemolyticus, type 27, by this method.

One of the two rabbits inoculated with 5 c.c. pericardial fluid died in 72 hours as a result of haemolytic streptococcal septicaemia. The serological examination of the strain, isolated in pure culture from the heart-blood, revealed that it was type 27. The remaining animal was apparently unaffected and no abnormality was detected when the rabbit was killed seven days later.

Case 3 : The direct broth culture of 0.5 c.c. pericardial fluid remained sterile after seven days' incubation at 37°C., but the pericardial exudate yielded Streptococcus haemolyticus, type 18, in pure culture.

One of the two rabbits inoculated with 5c.c. pericardial fluid died in five days as a result of septicaemia due to the same organism. The second

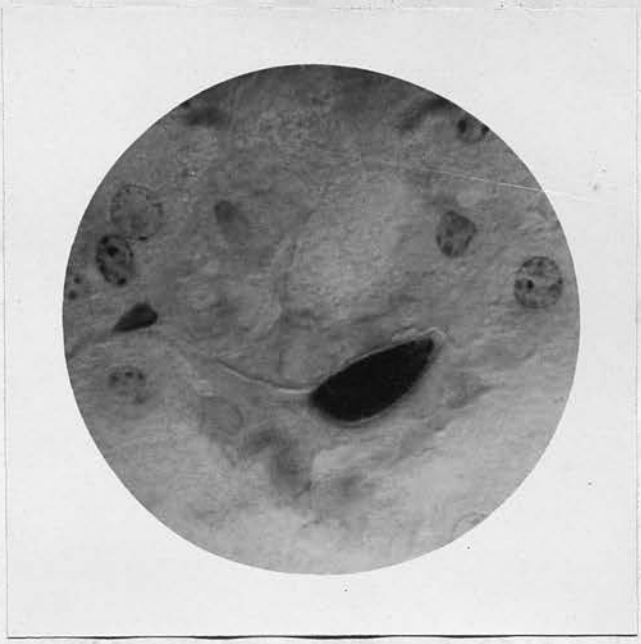

Fig. 1.- Kidney of rabbit five days after inoculation with pericardial fluid obtained at post-mortem from acute rheumatic pericarditis in human subject. Stained Gram X1000.
N.B. - Isolated group of streptococci, shown on culture to be of the beta haemolytic variety, seen between renal tubules. There is a complete absence of any surrounding phagocytosis, and the cocci are reddish-purple.

Fig. 2.- Kidney of rabbit five days after inoculation with pericardial fluid obtained at post-mortem from acute rheumatic pericarditis in human subject. Stained Gram X 1000.
N.B. Elongated group of tightly packed streptococci, shown on culture to be of the beta haemolytic variety, seen between renal tubules. The appearance is strongly suggestive of an intracellular multiplication of the organisms, and at one end of the group a cell nucleus (N) can be seen, surrounded by cocci which stain reddish-purple in colour.

animal survived until the 6th. day, but it was obviously ill and was then sacrificed. At post-mortem, the only abnormality noted was moderate enlargement of the spleen from which Str. haemolyticus, type 18, was recovered in pure culture.

Haemolytic streptococci in the tissues of experimental animals

In the tissues of the experimental animals from which haemolytic streptococci were recovered, a peculiar distribution of the organisms was occasionally noted. The morphology was unlike that usually associated with suppurative lesions due to this group of organisms. There was a tendency for the cocci to be clumped into large masses, the individual members of which were feebly Gram-positive, or frankly Gram-negative. Another curious feature was the lack of phagocytic reaction in the vicinity of the bacterial masses. Such an appearance is seen in fig.1, p.138 , which is a high-power view of rabbit kidney. Fig. 2, p.138 , illustrated a similar aggregation of organisms with a few polymorphs attracted to the site. Towards one end of this group, a cell nucleus can be seen, surrounded by cocci, suggesting that the mass effect had resulted from intracellular reproduction. This is well illustrated in fig. 3(p.140) which shows a polymorph, laden with cocci, entrapped in a small

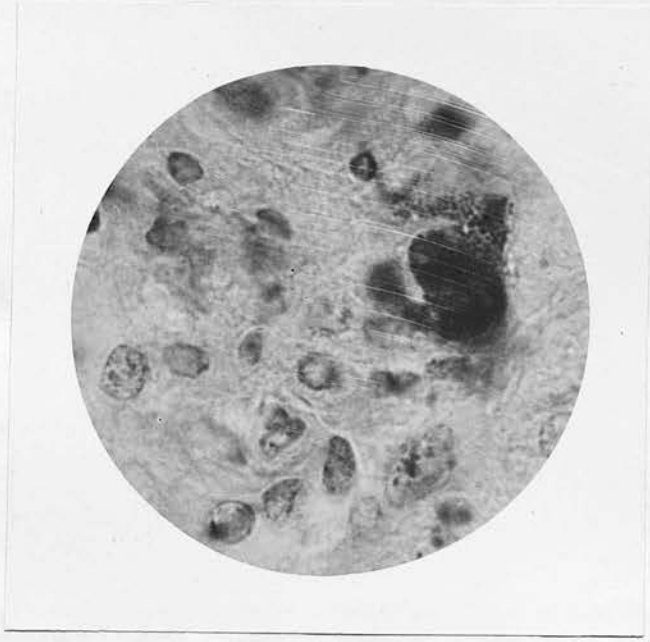

Fig. 3 - Rabbit kidney. A polymorphonuclear leucocyte, containing many streptococci, some of which have escaped from within the cell: entrapped in capillary of kidney glomerulus. Stained Gram X 1000.

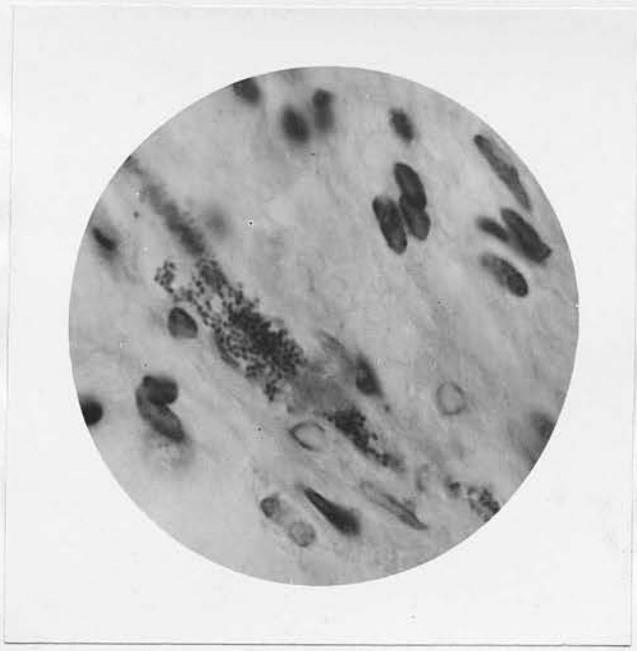

Fig. 4 - Rabbit kidney. Chains of cocci extend between the renal tubules, and phagocytic activity is more marked. Stained Gram X 1000.

capillary of the kidney glomerulus. Elsewhere in the tissues, the more usual distribution of streptococci was noted, as in fig. 4, (p.140), indicating that the masses previously described were true cocci.

Haemolytic streptococci in lymphatic tissues of man

As a matter of interest, the above appearances were compared with those observed during the stage of tonsillar infection in man. In fig. 5 (p.142) cocci are shown at the surface of the tonsil, and others within a polymorph. Sometimes intracellular proliferation of cocci was observed locally in the tonsil, as in fig. 6 (p.142) and this process occasionally reached exaggerated proportions as shown by fig. 7 and 8 (p.143).

Microscopic appearances in human heart valves

The comparison was extended to the microscopic appearances in human heart valves with vegetations, stained by the prolonged Gram method previously described. Fig. 9 and 10 (p.144) showed that similar reddish-purple coccoid bodies were to be found in such tissues both intra- and extracellularly. In fig. 11 (p.145), a low-power view of the centre of the base of a typical rheumatic vegetation, the type of cell reaction present is indicated. In this case, as in all others, the absence of any microscopic evidence of subacute bacterial endocarditis was confirmed.

Fig. 5 - Section of tonsil from fatal case of acute rheumatism. Cocci are seen lying intracellularly in a polymorph leucocyte. Stained Leishman X 1000.

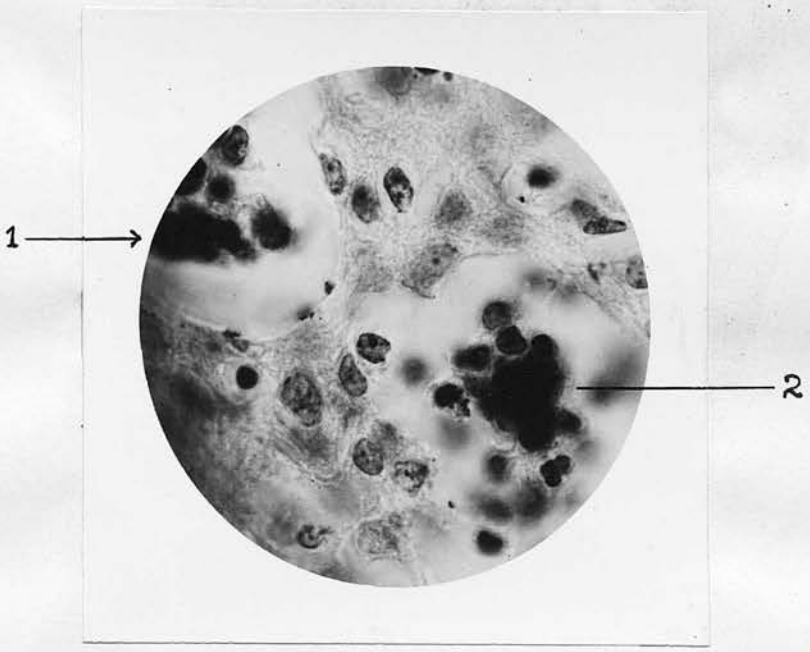

Fig. 6 - Section of tonsil from fatal case of rheumatism. Intracellular proliferation of cocci is seen at 1 and 2. Stained Leishman X 1000.

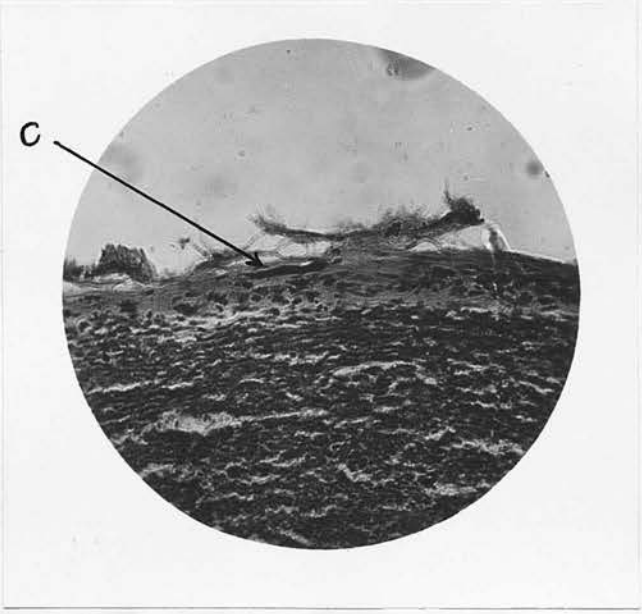

Fig. 7 - Section of tonsil. A dense mass of cocci(C), situated at base of tonsil, indicates extent to which aggregation of these organisms may occur. Stained Leishman X 200.

Fig. 8 - Higher magnification of mass of cocci shown in fig. 7. Only at the edge can individual members be discerned (K). Stained Leishman X 1000.

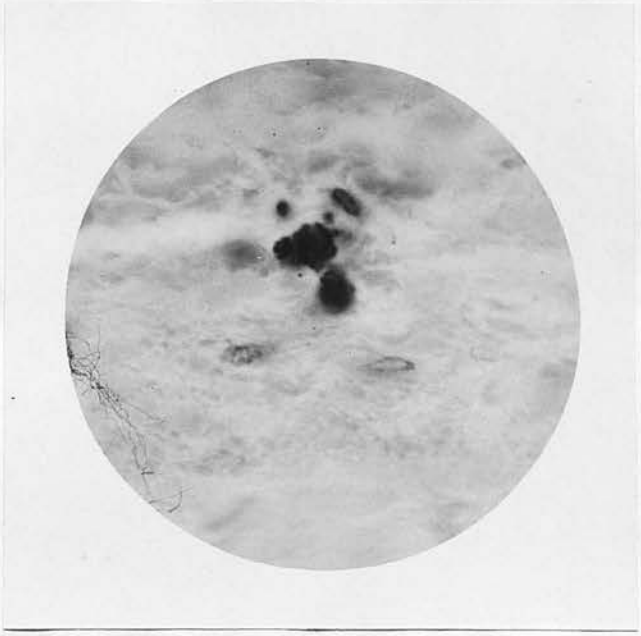

Fig. 9 - Section at base of human heart valve with rheumatic vegetations. Shows intracellular coccoid bodies, identical in appearance with intracellular cocci seen in previous photographs. Stained Gram X 1000.

Fig. 10 - Section of human heart muscle. An aggregated mass of bodies, similar in appearance to those of fig.1 is shown. Stained Gram X 1000.

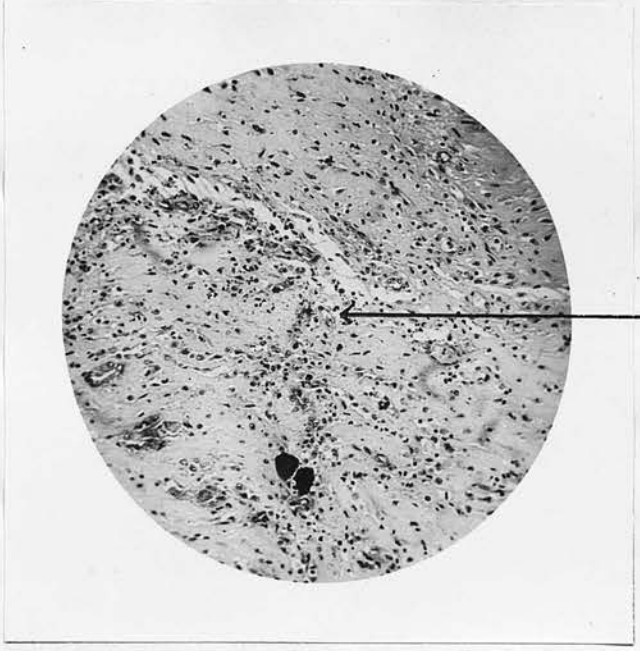

Fig.11- Section of human heart valve at base of rheumatic vegetation. Shows intracellular infiltration at base of valve. Serial sections through this valve revealed no indication of any surface lesion.
Stained Gram X 1000.

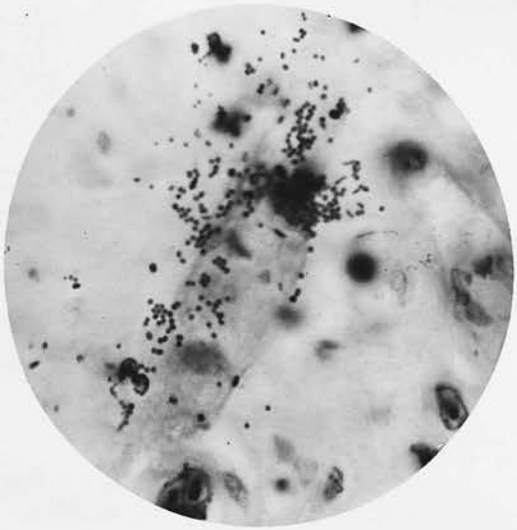

Fig.12- High-power view of area marked X at centre of rheumatic vegetation in fig.11. A group of coccoid bodies, distributed in pairs and in short chains is shown.
Stained Gram X 1000.

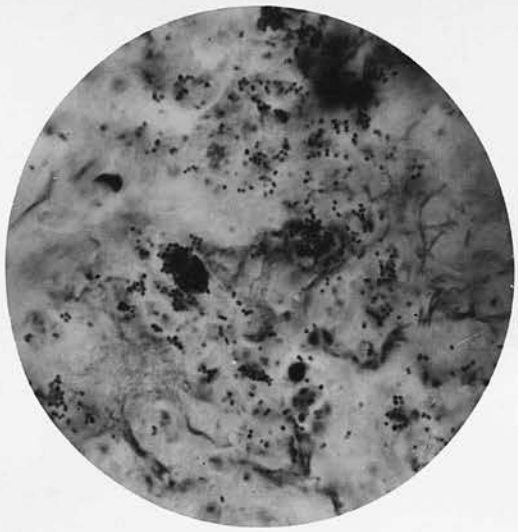

Fig.13- Experimental pericarditis in rabbit following haemolytic streptococcal inoculation. The strain used was that recovered from the cultures of valve of fig.12 and 13.
Stained Gram X 1000.

At the centre of this vegetation, the high-power appearances were those illustrated by fig. 12(p.145). The distribution of the bodies included pairing and short-chain formations. In the stained preparations, some bodies are Gram-positive and others Gram-negative. It is interesting to compare fig. 12 and 13. The latter was taken from an experimental pericarditis in a rabbit, following inoculation of the pericardial sac with the strain of haemolytic streptococcus isolated from the culture of the heart-valve shown in fig. 11. The similarity between the two pictures is striking. Despite the closest examination of many sections in this and other series, only one exhibited such well-marked features.

DISCUSSION AND CONCLUSIONS

The conclusions to be drawn from the comparison of human and experimental tissue appearances are not easily made. The bodies observed in the heart tissues may represent degenerated cocci or an alternative explanation for their presence is that they are derived from the tissues themselves e.g. mast-cell granules. The differentiation between the two is comparatively easy if the cocci react to staining methods and are distributed in the expected manner. It has been shown, however, that in experimental tissues, streptococci may be aggregated into masses and

differentiation under these circumstances becomes exceedingly difficult.

At present, final conclusions as to the nature of these bodies have not been made. In view of the frequency with which streptococcal tonsilitis has been shown to precede the onset of rheumatism, and of the pathological reaction known to be taking place in the tonsil, it is tempting to speculate whether some of these appearances in human heart valves may not actually be due to the presence of cocci, particularly as t the cultural results undeniably support the relationship between streptococcal infection and rheumatism. If that be the case, then they indicate the existence of a new and peculiar relationship between the haemolytic streptococcus and the human host.

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SECTION I

EPIDEMIOLOGY OF HAEMOLYTIC STREPTOCOCCAL INFECTION IN RELATION TO ACUTE RHEUMATISM

PAPER I

HAEMOLYTIC STREPTOCOCCAL EPIDEMIC AND FIRST APPEARANCE OF RHEUMATISM IN A TRAINING CENTRE

INTRODUCTION

The reawakening of the rheumatic process in patients as a result of respiratory infection, particularly tonsilitis, has been fully described by many observers, including Raven (1923), Boas and Schwartz (1926), Hiller and Graef (1928), Schlesinger (1930), Collis (1931) and Coburn and Pauli (1935).

Reports concerning the appearance of rheumatism in apparently healthy communities after such epidemics have still further stressed the linkage between the two conditions. Thus Glover and Griffith (1931) and Bradley (1932) described outbreaks in schools after epidemics of haemolytic streptococcal tonsilitis. The circumstances under which a similar outbreak occurred in a training centre are here recorded.

Conditions in the training centre Sd

The personnel of this establishment consisted partly of 188 apprentices, aged fifteen to seventeen years, divided into four classes of approximately fifty members, named A, B, C and D. Class D was the most advanced and had been together for the longest period, while classes C, B and A joined in that order. In addition, there were approximately 360 trained men, the majority being in the age-group 21-26 years.

The community was shifted as a unit to temporary quarters in the country in May, 1940. Near the end of September, 1940, the new apprentices in class A were added. Previously the members of this class had been at school or in work in separated areas, and a preliminary course of 3-4 weeks was their only experience as a unit.

The workshops and teaching rooms in the new quarters were reasonably adequate for the purpose, and were used by both apprentices and trained men. The two groups differed essentially in the type of living accommodation provided for their use. The trained men were housed in private billets whereas the apprentices were stationed in a large country house and a number of semi-permanent huts. The house was in good repair and provided messing, recreation and part of the dormitory accommodation, while the temporary huts supplied the bulk of the dormitory space. These huts had been placed on grass lawns beside the house but

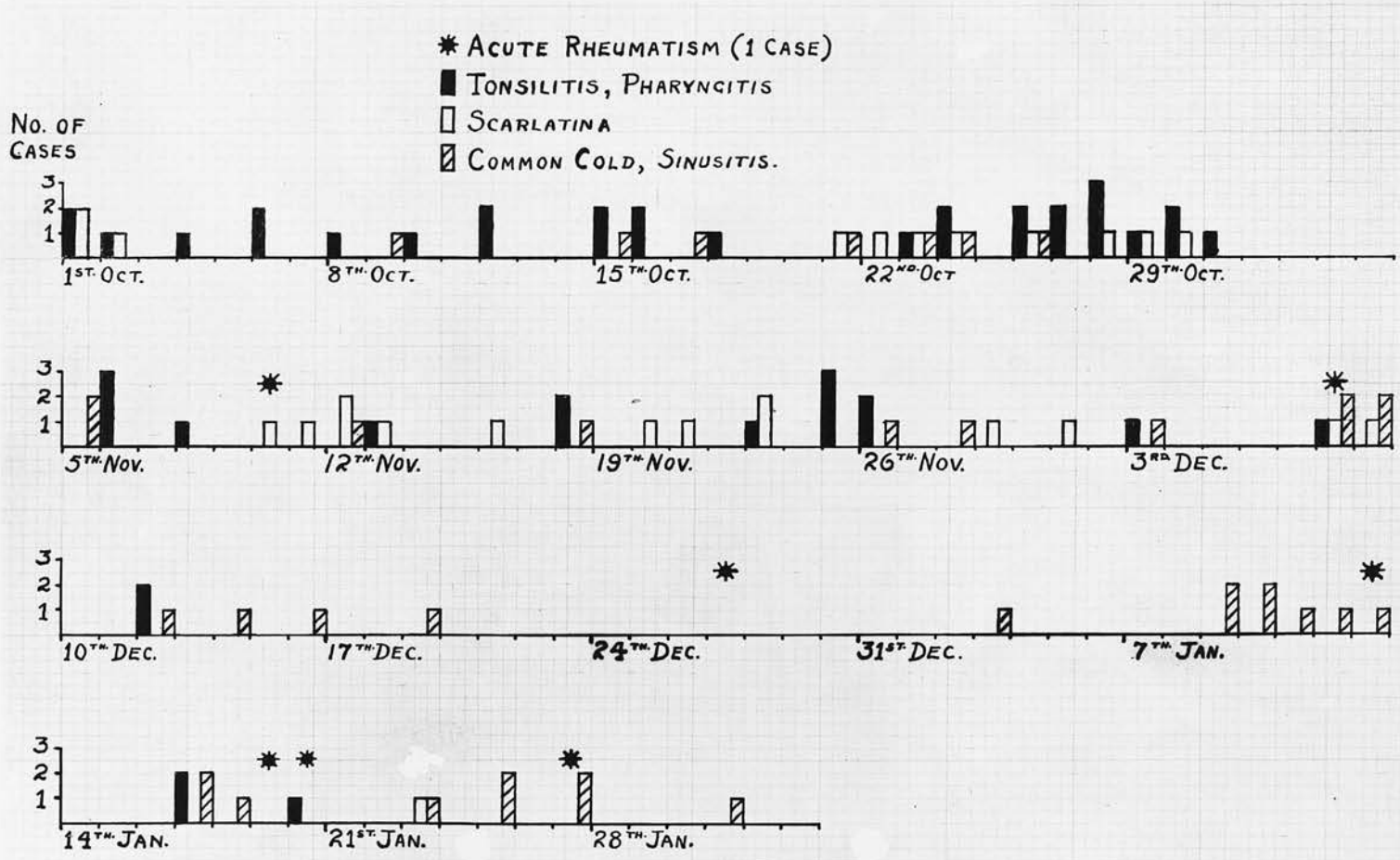

CHART 1 : TO SHOW THE DISTRIBUTION OF ALL NOTIFIED RESPIRATORY TRACT INFECTIONS IN RELATION TO TIME IN TRAINING CENTRE Sd DURING EPIDEMIC PERIOD.

adequate approach roads had not been provided. As a result of excessive rain, the huts became surrounded by heavy mud and were generally damp. Although there was no indication of overcrowding in the dormitories, the "black-out" sytem considerably restricted ventilation at night. The recreation hours of the trained men were spent either in the privacy of their billets or elsewhere away from the centre. The only recreation room for the apprentices was a living room in the house. This room was unsuitable for the numbers of apprentices who gathered there in the evening.

History of the outbreak

The health of the apprentices and other personnel during the summer months was excellent and relatively few cases of respiratory infection were seen. A survey of the records showed that only 12 cases of tonsilitis were treated in the sick-bay during the thirteen weeks of July, August and September. During the next twelve weeks of October, November and early December, 98 respiratory infections and complications were admitted, as follows:-

No. of cases	Diagnosis
20	Scarlatina
51	Tonsilitis
7	Sinusitis
8	Otitis media or externa
5	Bronchitis
7	Common cold

Chronological order of cases

Chart 1(p.150) shows the occurrence of all

notified respiratory tract infections in relation to time, and indicates that the epidemic included most of the clinical manifestations of haemolytic streptococcal infection. Cases of scarlatina, tonsilitis and secondary complications such as sinusitis were notified at irregular intervals, the greatest incidence being in the week ending 29th. October.

Class distribution of cases

The number of cases in relation to apprentice classes and trained men is shown in Table 1(p.153). The incidence of all conditions listed in the table among the trained men and apprentices was 64 and 474 per 1000 respectively over the period of observation. This marked difference emphasises the extent to which the apprentices bore the brunt of the epidemic. The different classes of apprentices suffered in inverse proportion to the length of their existence as a unit, the youngest class, A, experiencing the greatest number of cases. Not only did most of the primary infections occur in this class but complications such as otitis media were also more common. Class B presented the next highest incidence, and 78 per cent of all infections in apprentices were divided between these two younger classes.

Dormitory distribution of cases in apprentices

Classes A and B, with 6 exceptions, slept in the four dormitory huts, while classes C and D, with six members of class B, slept in the house. Classes A

TABLE 1

To show the distribution of cases in trained men and in classes of apprentices.

Diagnosis	Tot.No. Cases	Number of cases in (1)Apprentices:Class -					(2)Trained Men
		A	B	C	D	Tot.	
Scarlatina	27	14	8	4	0	26	1
Tonsilitis pharyngitis	56	22	6	7	4	39	17
Sinusitis	7	2	2	0	2	6	1
Otitis	8	6	1	1	0	8	0
Common Cold	9	5	0	0	2	7	2
Bronchitis pneumonia	6	2	2	0	0	4	2
Totals	113	51	19	12	8	90	23

and B each occupied two huts and, as shown in Table 1 (p.153), the respective totals for combined infections were 51 and 19. All but 4 of the 51 cases of scarlatina were in hut occupants.

Bacteriological examinations

Only a limited investigation was possible and consisted of the taking of throat swabs from all persons in the sick-bay at the time of visiting the institution, the results being collected in table 2, (p.155). Of the fourteen sick patients, ten had haemolytic streptococci in the throat flora. An indication of the probable extent of streptococcal infection was given by the three positive swabs from the four outpatients.

Dick-test results

All the apprentices were Dick-tested in the period Dec. 16th.-19th., 1940. The results are detailed in Table 3 (p.156). As was expected, there was a progressive reduction in the proportion of Dick-positive reactors in classes B, C and D, due to the differences in class-age. On the other hand, the proportion of susceptibles remaining in class A after the epidemic was only 36.8 per cent, which was even fewer than in the oldest class, D, with its 47.1 per cent. Unfortunately the pre-epidemic Dick reactions were unknown, but it was reasonable to suppose that the youngest and most-recently formed group, namely class A, contained at least as high a proportion of

TABLE 2

Results of examination of throat flora for haemolytic streptococci from cases and attendants in sick bay, together with four out-patient boys.

No. of cases	Diagnosis	Results.			
		-	+	++	+++
4	Tonsilitis	1	0	3	0
1	Otitis	1	0	0	0
1	Post-scarlatinal convalescent	1	0	0	0
1	Acute scarlatina	0	0	1	0
3	Sub-acute rheumatism	0	1	1	1
3	Injuries	1	1	1	0
1	Impetigo	0	1	0	0
4	Out-patients	1	1	0	2
18		5	4	6	3

TABLE 3

Table 3 : TO SHOW THE POST-EPIDEMIC DISTRIBUTION OF DICK REACTORS IN THE ARTIFICER CLASSES.

Class	Result of Dick Test		
	Positive	Negative	Pseudo-Reaction
A	36.8	60.5	2.7
B	65.3	32.6	2.1
C	52.7	41.6	5.7
D	47.1	52.9	0

positive reactors as the 65.3 per cent noted in class B, which was the next youngest group. The ultimate effects of the epidemic were therefore not entirely disadvantageous, for the antitoxin immunity of class A was raised above that of any other class.

Occurrence of acute rheumatism

At the time of writing, eight cases of acute rheumatism have been notified as follows:-

Case	Age in yrs	Designation	Class	Onset of Rheum. (1)	Previous illness (2)	Interval between (1) and (2) in days
O.C.	16	Apprentice	B	10/11	Scarlat. 1/10	40
N.F.	16	"	A	18/11	Tonsil. 28/10	21
H.S.	16	"	A	19/11	Tonsil. 12/10	38
H.F.	16	"	C	8/12	Pharyng. 1/10	69
C.J.	16	"	B	21/12	Scarlat. 12/11	50
P.B.	17	"	B	19/1	Nil	
L.G.	16	"	A	20/1	Scarlat. 12/11	69
H.E.	16	"	B	27/1	Scarlat. 29/11	59

Thus, all the cases were in apprentices and all but one had already been admitted to the sick-bay during the streptococcal epidemic with pharyngitis or scarlatina.

DISCUSSION

The investigation provided a good example of the circumstances under which a typical outbreak of haemolytic streptococcal infection developed in a healthy community with the subsequent appearance of acute rheumatism. It was unlikely that the recorded figures gave an indication of the full extent of the epidemic for the apprentices, as a group, were keen to keep abreast of their class-mates and were apt to conceal minor illnesses.

Factors which contributed to the outbreak were, firstly, the introduction of the susceptible class A apprentices at a time when the risk of infection was high. As suggested by Dudley (1926) this probably resulted in an increase in virulence of the infecting organism by passage. Following the increase in virulence, the number of clinical infections appearing in a given class varied with the age of the class and with the susceptibility of its members. Thus, almost 80.0 per cent of cases occurred in the younger classes, A and B. Contrary to expectation, the ratio of the number of cases of scarlatina to those of tonsilitis in class A was 1 : 1.5, and in class B was 1 : 0.75. This distribution suggested that the members of class A had experienced greater contact with infection prior to the epidemic than had those of class B.

The Dick test results showed that the influence

of the epidemic on the antitoxin immunity of class A was very considerable and, within a few weeks, produced an effect which would have occupied many months under normal circumstances.

The marked difference in the total incidence of respiratory infections, viz. 51, 19, 12 and 8 in classes A, B, C and D respectively, was a good example of the effect of herd immunity, the class of longest age showing the greatest percentage of survivors, irrespective of the individual immunity.

Undoubtedly, the second factor of importance was the lowering of resistance, induced partly by the damp conditions in and around the dormitory huts and the lack of adequate drying rooms. As a result, wet socks and boots were continuously worn during the working and school hours, for there was a general reluctance to put more than one pair of boots into daily use. Contributory factors included a tendency for the boys to spend too little time in sleep or rest after a day which was very fully occupied by a curriculum divided between practical and theoretical instruction.

The third factor was the high infection risk to which the apprentices were exposed as a result of the overcrowded state of the recreation room, and the close contact around the central heating stoves of dormitories.

Acute rheumatism did not appear until the streptococcal outbreak had been in progress for some time, and all save one case had been involved in that epidemic. Moreover, all cases of rheumatism occurred in apprentices and, with one exception, in the two classes which were most seriously involved in the epidemic. Environmental features common to all classes, such as dietary factors, climate, physical and mental stress, were thus completely overshadowed by the preceding streptococcal infection.

SUMMARY AND CONCLUSIONS

1. The circumstances attending an outbreak of haemolytic streptococcal infection in a training centre, followed by acute rheumatism, are described.
2. The incidence of streptococcal infections in trained men and apprentices was 64 and 474 per 1000 respectively.
3. 78.0 per cent of streptococcal infections occurred in members of the two younger classes of apprentices.
4. Eight cases of acute rheumatism were all in apprentices, seven cases being in the same two classes. All but one case had had tonsilitis or scarlatina during the epidemic.
5. The smallest percentage of post-epidemic Dick-positive reactors was found in the youngest class, which had suffered most in the outbreak.

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SECTION I CONTINUED

EPIDEMIOLOGY OF HAEMOLYTIC STREPTOCOCCAL INFECTION IN RELATION TO ACUTE RHEUMATISM

PAPER 2

EPIDEMIC RHEUMATISM

INTRODUCTION

Although multiple cases of acute rheumatism have been observed to follow waves of streptococcal infection in institutions (section I, paper 1)there is doubt as to whether acute rheumatism itself becomes epidemic under such circumstances, or whether the element of infectivity lies in the preliminary throat infections as concluded by Sheldon (1931) .

The occurrence of acute rheumatism in a training centre to an extent which reached epidemic proportions was therefore of interest.

EPIDEMIOLOGICAL OBSERVATIONS

Personnel in training centre Ac

The bulk of the personnel consisted of youths aged fifteen to seventeen years, who had all passed a routine medical examination on admission., The youths were divisible into two classes, viz. 300 apprentices

and 900 boys. Apprentices were added to the establishment in large classes of sixty to seventy youths every term, while boys were joined at the rate of thirty to thirty-five every week until the end of July, 1938, when recruiting stopped.

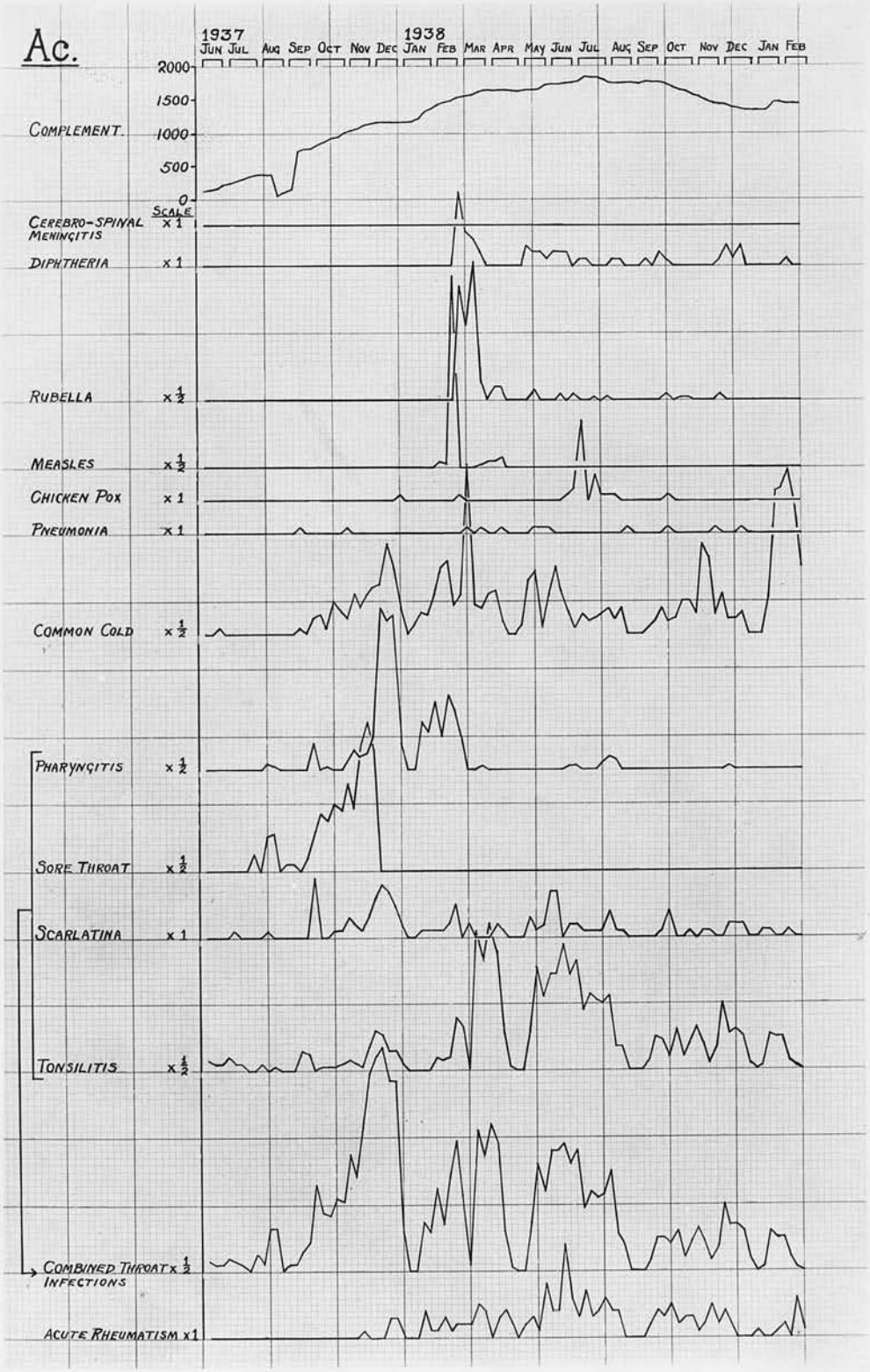
The establishment was opened in the spring of 1937 and the population steadily increased by the numbers of incoming recruits until the spring term of 1938, as shown in Chart 1(p.164). By that time, some of the boys had completed their training period of twelve months and had left the establishment. The average population during the period of observation numbered 1300, although the total entries were approximately 1900.

History of outbreak

Acute tonsilitis appeared in epidemic form in the autumn of 1937, and persisted throughout the 1937-38 session. In the earliest stages of the wave of streptococcal infection which preceded the appearance of rheumatism, the periodic examination of cases of tonsilitis and upper respiratory tract infection invariably indicated that haemolytic streptococci were responsible. Out of 126 swabs so taken, 112 were positive for this organism. The combined figure for throat infections was taken as a rough index of total streptococcal infection, and in the full period of observation this total reached 1466 cases while 162 cases of acute rheumatism and 132 cases of scarlatina

CHART 1

TO SHOW THE INCIDENCE OF VARIOUS INFECTIONS AND OF ACUTE RHEUMATISM IN TRAINING CENTRE Ac



were notified.

During the epidemic the severity of acute rheumatism varied from the hyperpyrexial polyarthritis of text-book description to less striking cases in which the initial diagnosis was difficult. Thus some cases had little or no fever and only transient indefinite joint pains, accompanied by an abnormal sedimentation rate and electrocardiographic changes. Some of the most severe cases of carditis occurred in boys whose illness was apyrexial throughout its course, while pericarditis developed in a few instances without premonitory symptoms or signs. The insistence of the clinical triad of fever, joint pains and carditis in each case would have resulted, therefore, in the exclusion of some of the most characteristic end-lesions of rheumatism. Accordingly, the criteria adopted for a diagnosis of acute rheumatism were, firstly, the evidence of acute infection as shown by pyrexia or increased sedimentation rate, followed by joint manifestations with or without carditis.

The monthly case returns for scarlatina, combined throat infections and acute rheumatism are indicated in Table 1 (p.167), and a pictorial representation of the epidemic is given in Chart 1 (p.169). The latter shows how the combined throat infections increased weekly from the beginning of the autumn term in 1937 until the dispersal of the community for Christmas leave. Towards the end of the term,

scarlatina showed a peak, although cases had occurred intermittently during the term. The first case of rheumatism was noted in the week ending 13th. November, but it was not until the end of the term that several cases appeared simultaneously. The marked reduction in the attack rate of throat infections due to dispersal was evident in the first two weeks of the spring term, but there was still a considerable number of cases. These rapidly increased as the term progressed and continued until the Easter vacation. Fewer cases of scarlatina occurred in this term, but cases of acute rheumatism were very numerous throughout, the total being 34. The summer term opened with an immediate recrudescence of streptococcal infection which reached its zenith early in the term and then became less intense. Scarlatina was again prevalent, particularly in the earlier part of the term. Rheumatism reached its greatest incidence during this term, the total number of cases being more than double that of the previous term. Chart 1 indicates that in the latter half of the term the disease became less frequent. The incidence of throat infections declined during the following terms and there was a corresponding reduction in acute rheumatism though many cases were still seen, probably as an after-effect of the preceding high rate of infection.

TABLE 1 :TO SHOW THE MONTHLY INCIDENCE OF ACUTE RHEUMATISM, SCARLATINA AND COMBINED THROAT INFECTIONS IN TRAINING CENTRE Ac FROM SEPT.,1937, TO FEB.,1939.

Month	Age of Centre in months	Acute Rheumatism	Scarlatina	Combined Throat Infections
Sept.	4	0	13	43
Oct.	5	0	6	113
Nov.	6	1	16	194
Dec.	7	6	25	193
Jan.	8	7	6	52
Feb.	9	7	16	104
Mar.	10	14	2	122
Apr.	11	8	1	51
May.	12	19	18	107
Jun.	13	27	13	142
Jul.	14	23	1	118
Aug.	15	2	1	17
Sep.	16	9	8	43
Oct.	17	14	5	46
Nov	18	13	2	39
Dec.	19	3	6	44
Jan.	20	3	2	33
Feb.	21	6	1	5
		162	132	1466

Incidence of rheumatism and scarlatina in relation to the period of admission

The effect of time on the incidence of rheumatism was noted as in Table 2(p.169). Details of the later cases were not available,but the majority were examined, including all those in the epidemic period. So far as acute rheumatism was concerned,it will be seen that the largest number of cases- 46.6 per cent- occurred in individuals within three months of entry. Of these, 18.4 per cent were in the first six weeks, and 28.4 per cent in the second half of the three month period.

During the epidemic in May, June and July,the proportion of new recruits, involved within six weeks of entry was increased, although many cases appeared in youths who had been in the institution for several months.

The total figures indicated that the chances of contracting rheumatism, in relation to the time of exposure to the environment, increased up to six months and then decreased.

On the other hand, 73.1 per cent of scarlatinal cases occurred within three months of entry. Of these, 54.8 per cent were in the first six weeks, and 18.3 per cent in the second half of the three months. The total figures indicate that the greatest risk of developing scarlatina was in the first dew weeks of entry, but that the risk still existed after several moths exposure.

TABLE 2

TABLE 2 : TO SHOW THE MONTHLY DISTRIBUTION OF SCARLATINA AND ACUTE RHEUMATISM IN RELATION TO THE LENGTH OF TIME AFTER ADMISSION TO THE TRAINING CENTRE Ac.

Month	Age of Centre mths.	No. of weeks between Admission and onset of Disease					Total No. cases per month
		1 - 6	7 - 13	14 - 26	27 - 39	40 - 52	
ACUTE RHEUMATISM							
Absolute Number of Cases							
Nov.	6	1	0	0	0	0	1
Dec.	7	3	3	0	0	0	6
Jan.	8	0	3	2	2	0	7
Feb.	9	0	2	3	2	0	7
Mar.	10	1	5	6	2	0	14
Apr	11	0	4	2	2	0	8
May	12	1	3	6	8	1	19
Jun.	13	9	4	7	5	2	27
Jul.	14	6	7	9	1	0	23
Aug. part	15	1	0	1	0	0	2
Total		21	32	36	22	3	114
Percentage Totals							
		18.4	28.0	31.6	19.3	2.6	100.0
SCARLATINA							
Absolute Number of Cases							
Sept.	4	9	3	1	0	0	13
Oct.	5	5	1	0	0	0	6
Nov.	6	10	3	3	0	0	16
Dec.	7	8	9	7	1	0	25
Jan.	8	1	2	1	2	0	6
Feb.	9	10	1	2	2	1	16
Mar.	10	1	0	1	0	0	2
Apr.	11	0	0	0	1	0	1
May	12	10	2	4	2	0	18
Jun.	13	9	2	2	0	0	13
Jul.	14	1	0	0	0	0	1
Aug.	15	1	0	0	0	0	1
Sept.	16	4	0	4	0	0	8
Total		69	23	21	12	1	126
Percentage Totals							
		54.8	18.3	16.6	9.5	0.9	100

Type of acute rheumatism

Clinical records were available for cases occurring up to the end of the summer term in 1938 when the acute rheumatic cases fulfilling the criteria already detailed for the establishment of a diagnosis, totalled 114. Of these 104 had pyrexia, 97 developed joint pains and 102 showed evidence of carditis, so that the majority exhibited the clinical triad of joint pains, pyrexia and carditis. When last surveyed, the position as regards the clinical features was as follows:-

No carditis	12
Temporary murmurs	27
Cases in which a mitral systolic murmur developed and has persisted in the absence of any other signs of organic damage	31
Death (cardiac failure)	1
Pericarditis	8
Aortic reflux	4
Mitral reflux (11 of these now appear to be developing stenosis)	26
Established mitral stenosis	11

Thus 89 cases exhibited one or other of the more grave lesions typical of rheumatic fever.

Incidence of rheumatism in the various divisions of the institution

The institution was divided into five divisions of which the apprentices formed division E, while the boys made up divisions A, B, C and D. Each division consisted of some 300 boys and was further sub-divided into groups of 50. The youths of one group slept in the same dormitory and had meals at the same tables.

The various groups of a division occupied, as far as possible, the dormitories of one section of the institution. The youths of one division worked as a unit so that there was a certain amount of separation of the divisions from each other. This particularly applied to the apprentices who worked during the day in a building remote from the living quarters. The divisions could not be considered, however, as entirely separate populations within the establishment.

In Table 3(p.172) are shown the incidences of tonsilitis, rheumatic fever and scarlatina in the divisions. In addition are shown the cases which occurred among boys who had recently joined but had not been aasigned to a division. A period of four to six weeks was spent in a preliminary course before admission to a division, The number of new entries and boys in preliminary courses averaged 196, but this was a variable figure.

It may be seen that rheumatic fever occurred in all the divisions with approximately the same attack-rate. The same was true of the streptococcal infections, save that scarlatina was much more frequent in the D and E divisions and in the preliminary boys. Beyond noting the high frequency of both rheumatic fever and streptococcal infections in divisions and groups, the examination of the distribution of cases rendered no further assistance in the correlation of incidence.

TABLE 3

TABLE 3 : TO SHOW THE DISTRIBUTION OF ACUTE RHEUMATISM, SCARLATINA AND UPPER RESPIRATORY INFECTION IN THE DIVISIONS AND GROUPS OF TRAINING CENTRE Ac.

Division	Group	Number of Cases					
		Rheumatism		Scarlatina		Respiratory Infections	
A	1	19	1	5	1	178	35
	2		1		2		43
	3		6		1		36
	4		4		0		39
	5		1		1		20
	6		6		0		14
B	1	21	2	5	1	243	53
	2		2		0		54
	3		0		4		61
	4		3		0		33
	5		6		0		23
	6		8		0		19
C	1	17	3	11	1	212	55
	2		5		8		58
	3		2		0		34
	4		1		0		21
	5		0		1		27
	6		6		1		17
D	1	16	2	17	2	246	55
	2		3		4		75
	3		3		2		48
	4		3		3		25
	5		3		5		28
	6		2		1		15
E	1	22	1	26	5	225	22
	2		5		7		29
	3		3		1		34
	4		4		4		55
	5		3		7		47
	6		6		2		38
Preliminary Boys not assigned to a division		19		48		450	

Incidence of acute rheumatism and recruiting locality

Youths were drawn to the establishment from all over England, but the majority were from the North Midlands and from Scotland, as shown in Table 4 (p.174) which includes all admissions to the end of August, 1938.

The incidence of rheumatism in youths from certain grouped areas is shown in Table 5 (p.175). Northumberland and Durham were considered as one area on account of the similarity in the economic and industrial conditions in the two counties, and because many of the youths came from intervening districts. The heavy incidence amongst the boys from Tyneside was significantly greater than that of the remainder, as the following analysis shows:-

All admissions:

Total number of youths.....1806

" " " cases of rheumatism..... 114

Therefore, case incidence per 1000.....63.0

Tyneside admissions only:

Total number of youths..... 385

" " " cases of rheumatism.....39

Therefore, case incidence per 1000..... 101.3

All admissions omitting those from Tyneside

Total number of youths.....1421

" " " cases of rheumatism.....75

Therefore, case incidence per 1000.....52.8

TABLE 4

TABLE 4 : TO SHOW THE NUMBERS OF YOUTHS ADMITTED TO TRAINING CENTRE Ac FROM VARIOUS LOCALITIES FROM MAY,1937,TO AUGUST,1938.

Durham	261	Leicester	143
Lancashire	195	Dumfries	
Yorkshire	151	Mull and Argyll	
Lanark	134	Wigtown	
Northumberland	124	Donegal	
Lothian	95	Tyrone	
Antrim	85	Suffolk	
Aberdeen	66	Buckingham	
Fife	58	Warwick	
Hampshire	55	Clare	
Forfarshire	43	Monmouth	
Devon	38	Stafford	
Cornwall	36	Oxford	
Cumberland	31	Kinross	
Ayrshire	21	Peebles	
Renfrew	18	Elgin	
Surrey	18	Caithness	
Nottingham	17	Kincardine	
Londonderry	16	Dumbarton	
Cork	16	Selkirk	
Lincoln	14	Kirkcudbright	
Derby	14	Monaghan	
Perth	14	Gloucester	
Berwick	13	I. of Man	
Co. Down	12	Middlesex	
Glamorgan	12	Roxburgh	
Dublin	11	Wexford	
Essex	11	Sligo	
Kent	11	Flint	
Somerset	11	Hereford	
Dorset	11	Westmoreland	
Stirling	11	Wiltshire	
Sussex	10	Northampton	
Denbigh	10	Norfolk	
Ross and Cromarty	10	Pembroke	
Inverness	10	Sutherland	
		Nairn	
		Banff	
		Skye	

TABLE 5

TABLE 5 : TO SHOW THE INCIDENCE OF ACUTE RHEUMATISM IN YOUTHS RECRUITED FROM CERTAIN GROUPED AREAS.

District	Number of Youths	Acute Rheumatism	
		Number of cases	Percentage of Youths from the district
Northumberland and Durham	385	39	10.13
Lancashire	195	9	4.61
Yorkshire	151	9	5.96
Lanarkshire	134	6	4.47
Non-Industrial Areas	937	51	5.44

Therefore, the incidence in Tyneside youths was almost double that in the remainder. The next highest incidence was recorded in the youths from Yorkshire, but there was no significant difference from the remainder, the respective figures per 1000 being 59.6 and 63.6.

The reasons for the above finding are controversial, but it may be recalled that Northumberland and Durham show the highest mortality rate in the country, as shown by the following extract from the Registrar General's Report for 1931 :-

Mortality Rates for Male Lives

Age	England and Wales	Northumberland and Durham	Scotland	Glasgow
16	0.00227	0.00319	0.00232	0.00303
17	0.00259	0.00366	0.00262	0.00334
18	0.00284	0.00405	0.00289	0.00352
19	0.00332	0.00433	0.00310	0.00367
20	0.00316	0.00457	0.00326	0.00379

As is well-known, Tyneside was for years one of the worst of the "depressed" areas, and the high incidence of rheumatism in recruits from this area was probably a reflection of abnormal economic and environmental circumstances.

DISCUSSION

The occurrence of an outbreak of acute rheumatism of epidemic proportions in a community which had been recently surveyed and considered healthy, has been shown to be correlated with widespread infection by haemolytic streptococci. As regards the streptococcal aetiology of rheumatism, it is important to consider to what extent primary infection was encountered, as contrasted with a recrudescence of an existing lesion. It is a reasonable assumption that obvious rheumatic stigmata were eliminated at the entrance examination. Clinical experience has shown that latent infection readily escapes detection at such an examination, no matter how thoroughly it is conducted. However, it was most unlikely that all the cases which subsequently appeared were of this type. An attack rate of 63 per 1000 was almost certain evidence that primary infections were taking place. Granted that this was the case, then the association with haemolytic streptococcal infection assumed much greater significance. That this correlation was not exact did not, in itself, exclude streptococcal infection as a primary factor in the causation of rheumatism. Considering the analogy of another disease, which may be sporadic or epidemic in distribution and also spread by droplet infection, namely cerebro-spinal fever, the same absence of correlation between the incidence of cases and known contact

with the causative agent has been noted, Thus Dudley (1934) showed that a carrier-rate of 50 per cent for agglutinable N. meningitidis persisted for over twelve months, but no cases of cerebro-spinal fever appeared in a large community. On the other hand, in another community in which there were six cases of meningitis, the carrier-rate was only 5 per cent. By contrast, Glover (1920) had found that the carrier-rate was in the region of 70 per cent when cerebro-spinal fever outbreaks were imminent or present. There is apparently no simple rule governing the relationship between carrier-rates and the appearance of clinical cases. One of the varying factors which complicates the issue is the herd immunity of the exposed population. Although analogy is a dangerous form of reasoning, the possibility that a similar sequence is in operation when rheumatism follows streptococcal infection must be considered.

Of particular importance was the observation that a significantly higher proportion of the cases occurred in boys from the Tyneside area. In the absence of notification returns for rheumatism, the incidence in boys from this area as compared with the rest of the country is not known. As there is known to be significantly greater infection with B.tuberculosis in this community, the assumption is that rheumatism will also be more frequent. The higher proportion of cases among boys from the Tyneside area

may therefore be explained by a greater degree of latent infection. For the reasons stated it was unlikely that more than a fraction of the difference could be explained in this way. The additional factor was most likely to have been the greater susceptibility of Tyneside youths when exposed to an environment suitable for contracting infection.

The shape of the term curves for combined throat infections and scarlatina in Chart 1(p.164) suggested the following hypothesis. The steady upward climb of the former in the first term was an index of the increasing virulence of the haemolytic streptococcus as a result of passage through the community. This culminated in the appearance of multiple cases of scarlatina. Despite the reduction in incidence at the start of the second term, due to the dispersal of the population, the epidemic rapidly regained its former strength by reason of the increased virulence of the infecting strains. The "weeding out" of the scarlatinal susceptibles during the first term was indicated by the lowered figures in the second term. At the start of the third term, the increased virulence maintained the numbermof clinical cases at a high level, but an important antagonising factor came into play, namely, the increasing immunity of the community. Although scarlatina was more frequent in this term, the majority of the cases were in new entries who had not experienced the immunising action of the previous

terms.

The shape of the rheumatism curve was quite different, and suggested that the incidence of this condition was influenced by the immune state of the community. By the summer of 1938, the community was thoroughly saturated with haemolytic streptococci. The attack rate was so high that few persons escaped one attack of naso-pharyngeal infection. With few exceptions, all those who developed rheumatism had two or more attacks of throat infection before the onset of rheumatism.n In some the second attack of throat infection coincided with the appearance of joint manifestations.

A marked difference in the incidence of rheumatism and scarlatina,in relation to the time of exposure to the environment of the institution, was demonstrated. This again indicated that a different mechanism was at work if the haemolytic streptococcus was alone responsible for acute rheumatism as well as scarlatina. An analogous difference was observed in the incidence of scarlatina and tonsilitis, which was known to be of haemolytic streptococcal origin in the majority of cases. The latter difference was due largely to the variation in antitoxin immunity of the exposed herd as a result of the periodic introduction of fresh susceptibles. It is interesting to note that the curve for rheumatism in Chart 1(p.164)was much closer to that of tonsilitis than was the curve for scarlatina.

CONCLUSIONS

1. A description is given of an epidemic of upper respiratory tract infection, due to Str. haemolyticus, in a semi-closed community. The epidemic was followed by multiple cases of acute rheumatism.

2. In the period of observation, the average population was 1300, although the total entries were approximately 1900 : 1466 cases of throat infection occurred, which included 132 cases of scarlatina : 162 cases of acute rheumatism were notified in the same period.

3. Rheumatism in youths recruited from Tyneside was significantly more frequent than in the rest of the community.

4. Of the scarlatinal cases, 73.1 per cent developed within three months of entry, 54.8 per cent being in the first six weeks and 18.3 per cent in the following six : 46.4 per cent of rheumatic cases were notified within three months of entry, only 18.4 per cent being in the first six weeks and 28.4 per cent in the following six.

5. This difference in distribution is discussed in relation to the mechanism of acute rheumatism.

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SECTION I CONTINUED

EPIDEMIOLOGY OF HAEMOLYTIC STREPTOCOCCAL INFECTION IN RELATION TO ACUTE RHEUMATISM

PAPER 3

COMPARATIVE INCIDENCE OF VARIOUS INFECTIONS AND ACUTE RHEUMATISM IN CERTAIN TRAINING CENTRES

INTRODUCTION

Longstaff (1904) drew attention to the fact that waves of streptococcal infection, such as erysipelas, scarlatina and puerperal fever, synchronised with rheumatic fever. Atwater (1927) came to the same conclusion and, in addition, he noted the correlation between the mortality rates of the same conditions.

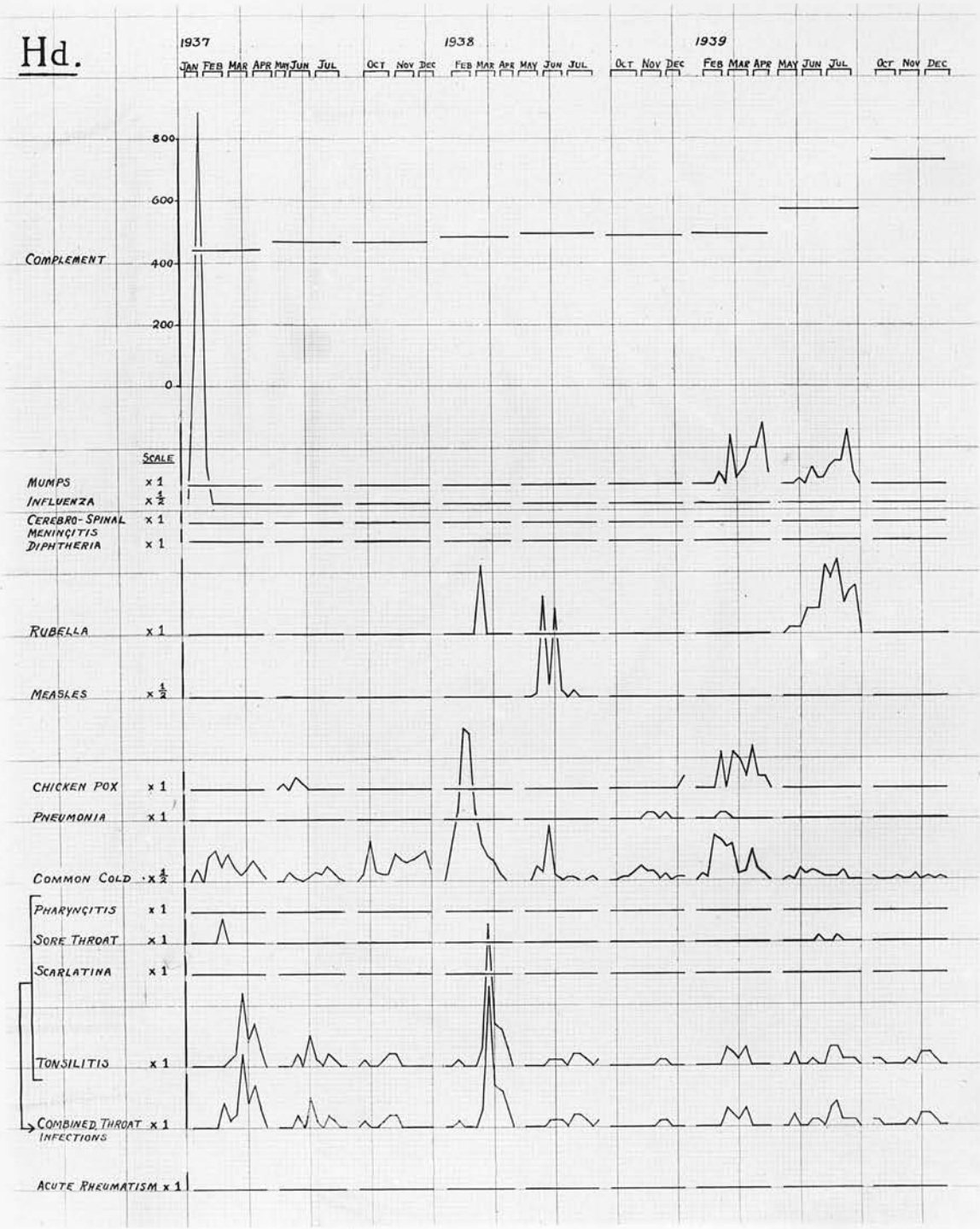
Reviewing the position of acute rheumatism in the Forces, Glover (1930) observed that the incidence varied as the pressure on sleeping accommodation. He drew an analogy between acute rheumatism and cerebrospinal meningitis, on the grounds that waves of acute tonsilitis preceded the appearance of cases of both diseases. Thus, a tonsilitis epidemic involving 175 per 1000 of the total personnel of 3530 was followed by 41 cases of rheumatic fever of whom 17 had carditis.

Dudley (1926) also noted the effect of overcrowding, but considered that damp and chill were also factors in determining the appearance of rheumatism. This conclusion was based on the fourfold drop in the incidence at a training centre after an improvement in hygiene administration which was especially directed to prevent damp and chill. Other factors demonstrated by Dudley to be of prime importance in influencing the incidence of infectious diseaae, including rheumatism, were the rate of change of population in training centres occasioned by the system of recruiting and discharging, and the herd immunity of the community. In Dudley's report, Vickery was quoted as observing that the incidence of disease fell more heavily on the newly-joined boys. Thus in 1912, there were 2949 entries on the sick-list of one training centre, of which 60 per cent were boys in their first three months, 24 per cent in their second and 16 per cent in the last three months of their training. Similar findings are detailed in section I, papers 1 and 2, in regard to haemolytic streptococcal epidemics and acute rheumatism, and attention was drawn to the longer exposure required before the maximum incidence of the latter disease was obtained.

The subject is now considered on a wider basis by examining the incidence of rheumatism and other diseases in several training centres which showed marked variation in morbidity rates.

CHART 1

TO SHOW THE INCIDENCE OF VARIOUS INFECTIONS AND OF ACUTE RHEUMATISM IN TRAINING CENTRE Hd.



EPIDEMIOLOGICAL OBSERVATIONS

The weekly records of sickness in various training establishments are graphically presented. The figures for tonsilitis, sore throat, pharyngitis and scarlatina have been combined to give a total figure because one or other of these terms had been used to describe the same condition in different centres.

Training centre Hd

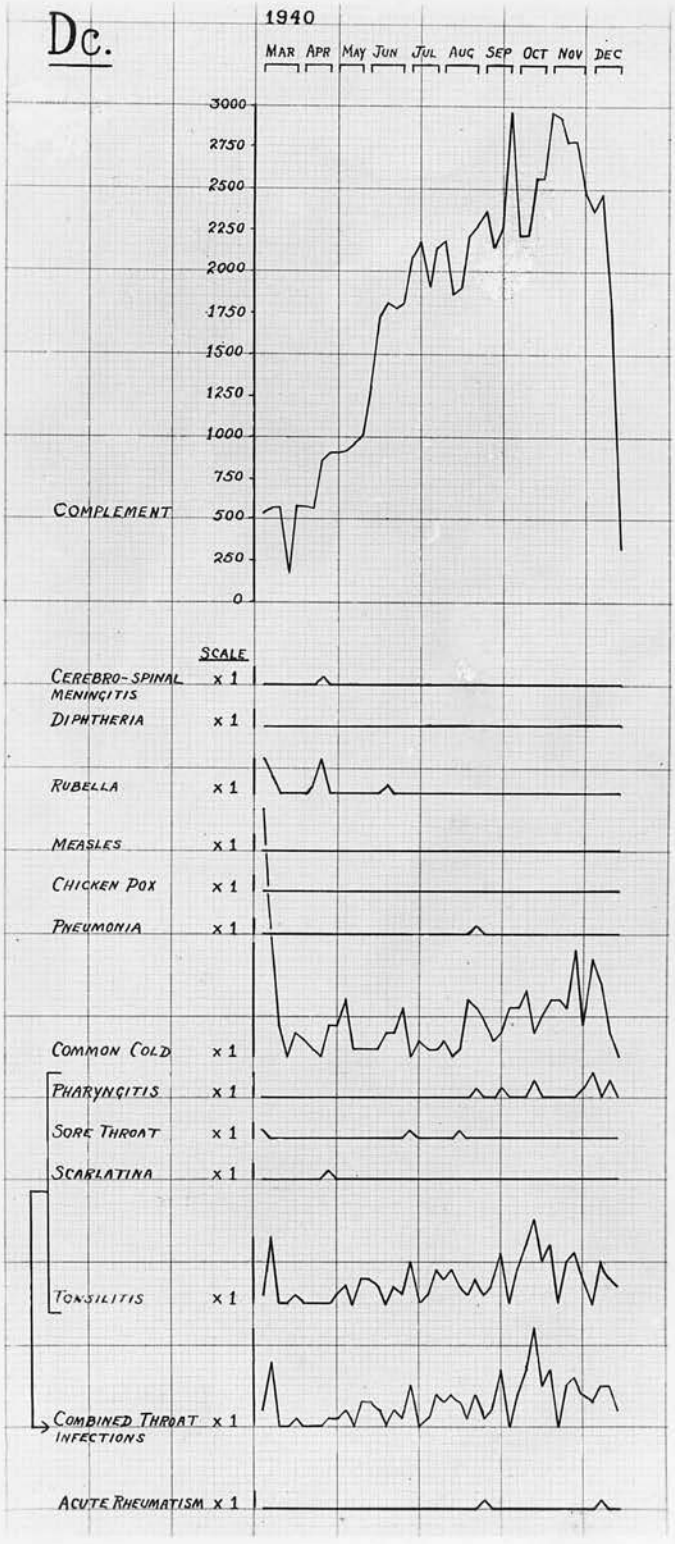
The period represented in Chart 1 (p.184) includes 1937, 1938 and 1939. Recruits were entered at the commencement of terms and were aged 14 to 16 years. The complement remained fairly steady until May and September of 1939 when two large increases were made. Acute rheumatism was entirely absent, but various epidemics of other diseases were experienced as follows:-

Disease	Term	Year
Rubella	Spring	1938
	Summer	1939
Mumps	Spring	1939
	Summer	1939
Measles	Summer	1938
Chicken Pox	Spring	1939
Common Cold	Spring	1938
	Spring	1939
Tonsilitis	Spring	1938
	Spring	1939

As the combined total of throat infections shows, there was little to note apart from the two seasonal epidemics. Dick immunisation was practised

CHART 2

TO SHOW THE INCIDENCE OF VARIOUS INFECTIONS AND OF ACUTE RHEUMATISM IN TRAINING CENTRE Dc.



and accounted in part for the absence of scarlatina in a susceptible age-group.

Training centre Dc

The period represented in Chart 2 (p.186) was the first ten months of the existence of the unit. Recruits were added at short intervals in varying numbers, so that the complement was raised from 500 to 3000 during the year. The recruits were of an older age-group than in the centre previously described, the majority being 20 to 24 years.

Apart from the epidemic of common cold at the start of the graph, the health of this centre was excellent and the incidence of rheumatism negligable, despite the heavy recruiting of the year.

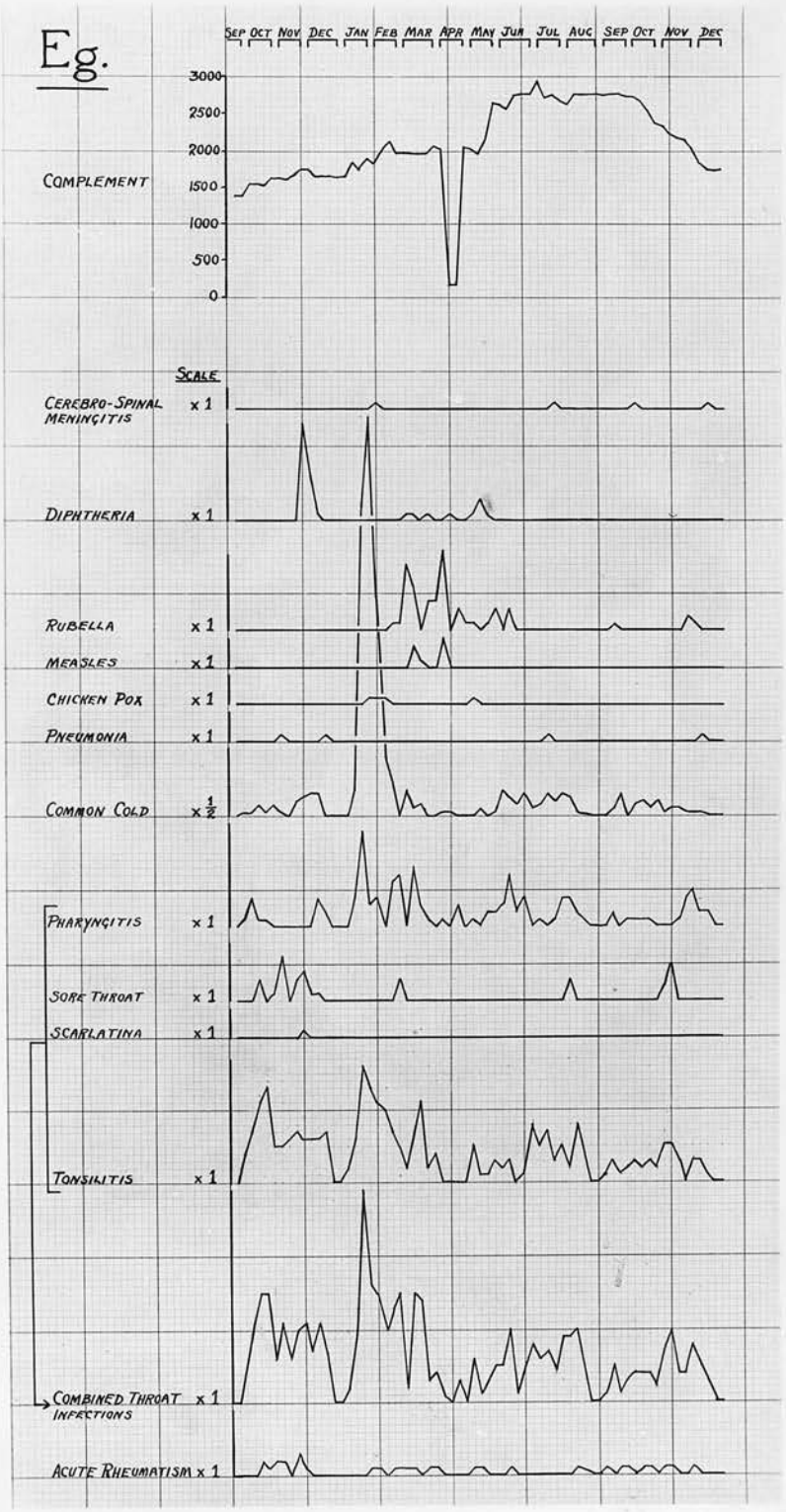
Training Centre Eg

The period represented in Chart 3 (p.188) covers the autumn term of 1939, when the establishment was opened, and all 1940. The recruits were aged fifteen to seventeen years, and the addition of new entries at short intervals raised the complement from an initial 1400 to approximately 2750 in 1940. In the last term the complement dropped rapidly to 1750.

Throat infections were moderately frequent during the observation period, the greatest number accompanying the epidemic of common cold in the spring of 1940. The estimated incidence of combined throat infections in 1940 was 140 per 1000, and 8 per 1000 of acute rheumatism.

CHART 3

TO SHOW THE INCIDENCE OF VARIOUS INFECTIONS AND OF ACUTE RHEUMATISM IN TRAINING CENTRE Eg.



Training centre Tv

The period represented in Chart 4 (p.190) includes the autumn term of 1938, together with the spring and summer terms of 1939. Recruits were entered at short intervals in small groups. The complement was approximately 750, but gradually fell as a slightly larger number of trained personnel were discharged at the time of admission of new recruits.

Acute rheumatism had been prevalent in this centre for several years, and during the year of observation an estimated incidence of 34 per 1000 was noted. Epidemics were experienced as follows:-

Disease	Term	Year
Rubella	Summer	1939
Common Cold	Spring	1939
	Summer	1939
Tonsilitis	Summer	1939

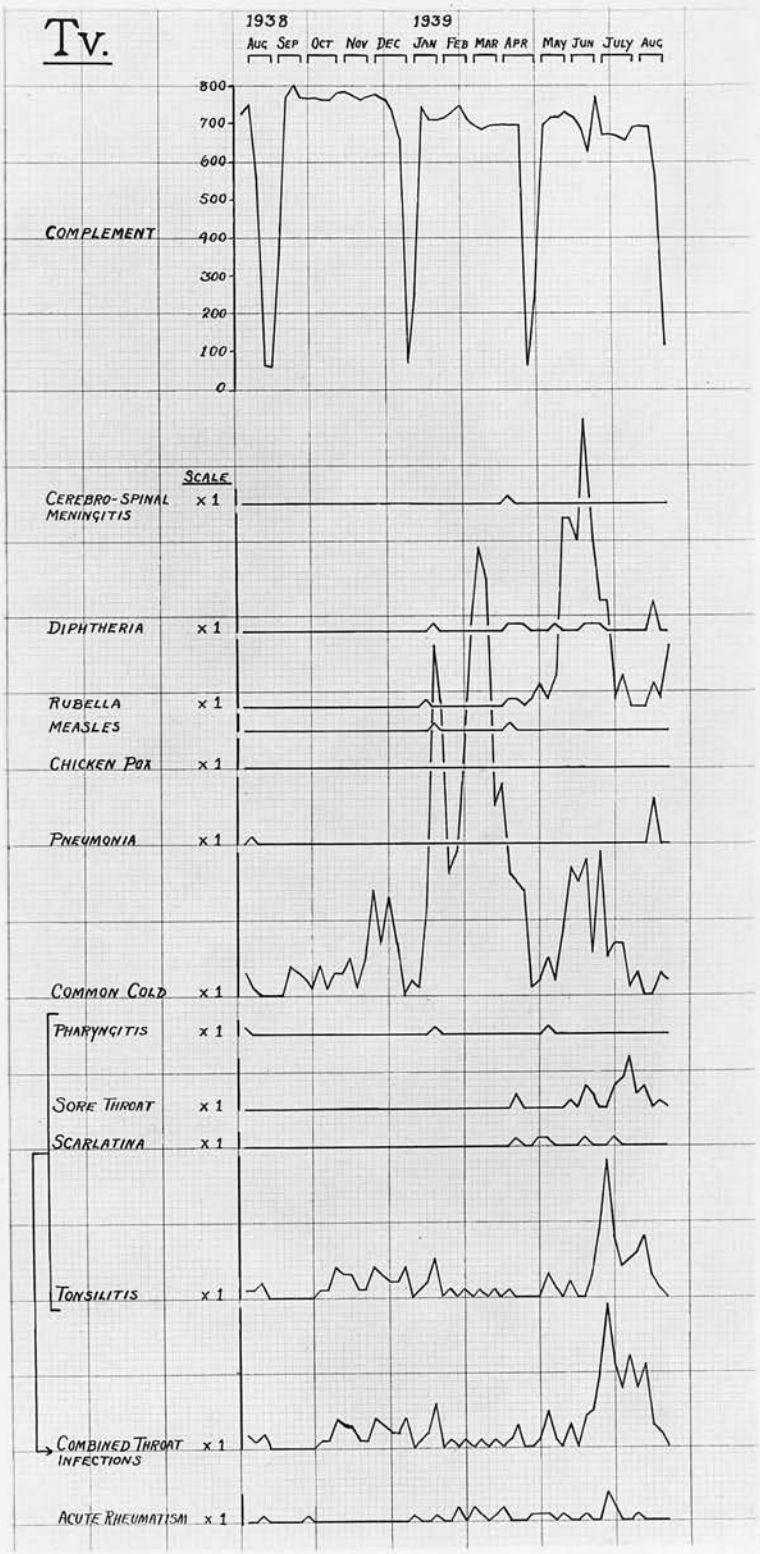
While no exact parallelism between the incidence of any two diseases can be distinguished, it may be noted that in the summer term of 1939, seven cases of acute rheumatism were notified in the two weeks of the first wave of a minor epidemic of tonsilitis which unexpectedly developed. In this summer term, ten cases of acute rheumatism occurred as compared with three cases in the preceding autumn term, and eleven cases in the intervening spring term.

Training centre Sg

The period represented in Chart 5 (p.192) covers the years 1938, 1939 and 1940. Recruits were aged

CHART 4

TO SHOW THE INCIDENCE OF VARIOUS INFECTIONS AND OF ACUTE RHEUMATISM IN TRAINING CENTRE Tv.



fifteen to seventeen years, and the complement approximated to 2100, with slight variations, until the spring term of 1940. The personnel was not fixed, but entries and discharges were made at short intervals, as in the last establishment. In the spring term of 1940, new entries were stopped but discharging continued so that the complement dropped. The personnel were finally transferred elsewhere in 1940 (May).

In the past few years, rheumatism has been common in this centre. In the three terms of 1939, 46 cases were notified, giving an estimated incidence for the year of 22 per 1000.

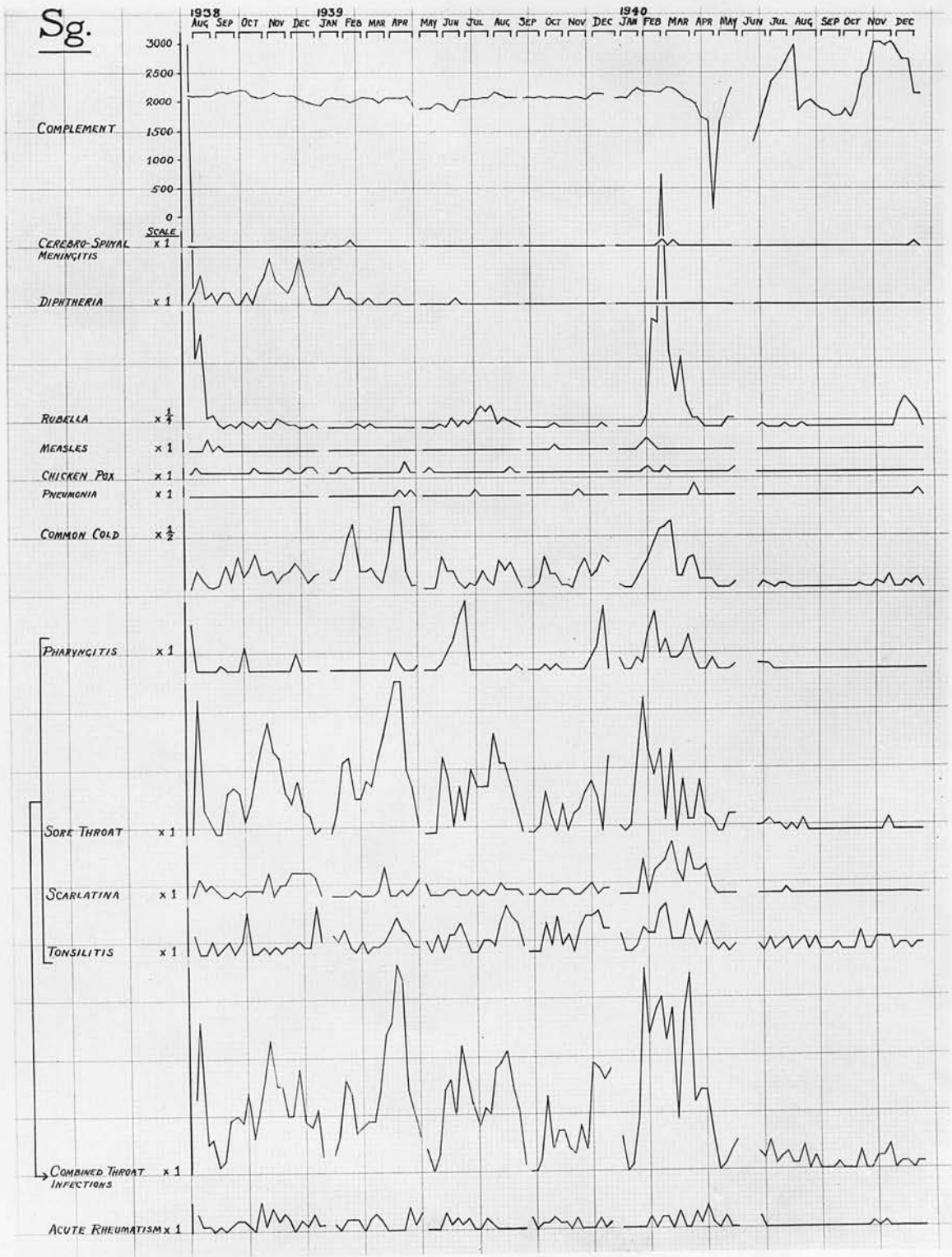
Epidemics were experienced as follows:-

Disease	Term	Year
Diphtheria	Autumn	1938
Rubella	Autumn	1938
	Spring	1940
Sore throat	Spring	1939
	Spring	1940
Scarlatina	Autumn	1938
	Autumn	1940

The prevalence of haemolytic streptococcal infection was demonstrated by the occurrence of cases of scarlatina over the entire period, in addition to the two epidemics. Acute rheumatism was similarly distributed over every term and, although there was no exact parallelism, there was a closer similarity between the curves for acute rheumatism and combined throat infections than for any other two conditions.

CHART 5

TO SHOW THE INCIDENCE OF VARIOUS INFECTIONS AND OF ACUTE RHEUMATISM IN TRAINING CENTRE Sg.



Thus the lowest figures for acute rheumatism were recorded in the autumn and summer terms of 1939 when throat infections were less frequent.

In Chart 6 (p.194) the term totals for rheumatism, scarlatina and combined throat infections are contrasted. It will be seen that in the first three terms, throat infections diminished to a slight extent, whereas scarlatina showed a marked reduction which probably indicated the increase in herd immunity against the toxic manifestations of haemolytic streptococcal infection. Acute rheumatism presented an equally steep fall during these three terms. In the last term the combined infection figures almost trebled those of the previous term and scarlatina increased sevenfold. Reference to Chart 5 (p.192) will show that the maximum complement for trainees aged fifteen to seventeen years was reached in this term, after the admission of large numbers of new recruits. Hence the sudden reappearance of multiple cases of scarlatina. There was a significant increase in rheumatism during this term, though not to an extent comparable with that of scarlatina.

Effect of change in personnel

Trainees were readmitted to this centre in June, 1940, but they were of an older age-group, being twenty-one years or over. The complement varied greatly during the remainder of the year, but the mean population was approximately equal to that of

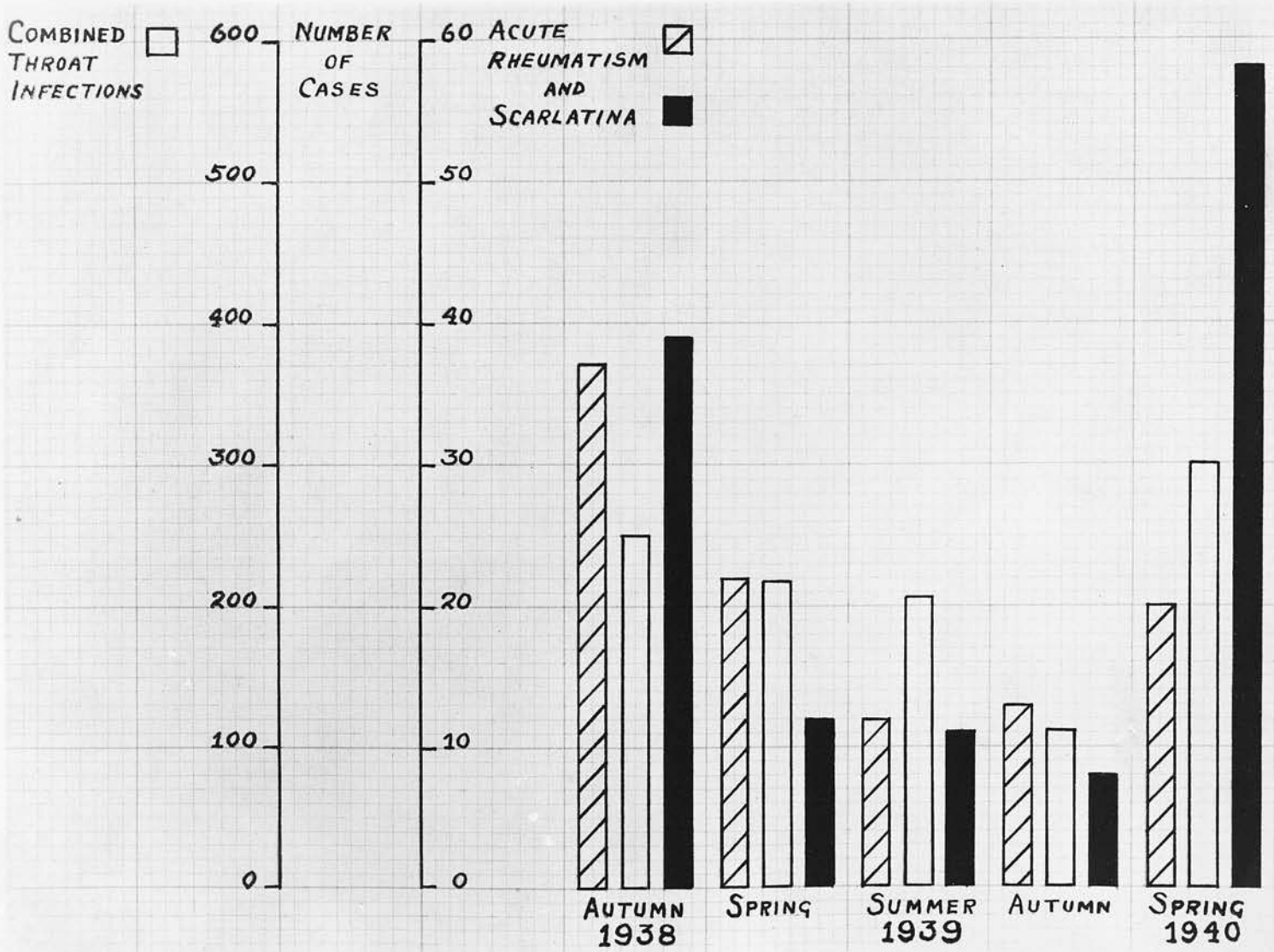


CHART 6 : TO CONTRAST THE TERM INCIDENCE OF COMBINED THROAT INFECTIONS, SCARLATINA AND ACUTE RHEUMATISM IN TRAINING CENTRE Sg.

the previous two years when the trainees were aged fifteen to seventeen years. Chart 5 (p.192) shows the significant and immediate reduction in the incidence of all respiratory diseases which accompanied the change-over in personnel. Only two cases of acute rheumatism were notified in the following seven months as against twenty-two and thirty-seven cases in the same months in 1938 and 1939. The point is further illustrated in Chart 7 (p.196) which contrasts the total number of cases of rheumatism, scarlatina and combined throat infections over similar periods in 1938, 1939 and 1940. The high rheumatism rate in 1938 and 1939 had been maintained for several years, and there was no reason to suppose that the dramatic reduction in 1940 would have been witnessed had the younger age-group continued in residence. This group was actually sent as part of the complement in the centre Eg, which had an estimated incidence of 8 per 1000 for acute rheumatism in 1940, but the rate in Sg youths only was not available. Conditions in training centre Sg were the same for both age-groups, and the observed difference in incidence of rheumatism was certainly significant.

Training centre Ew

The period represented in Chart 8 (p. 197) covers the autumn term of 1938, all 1939, and the spring term of 1940. The complement averaged 550, except in the last two terms. The recruits were

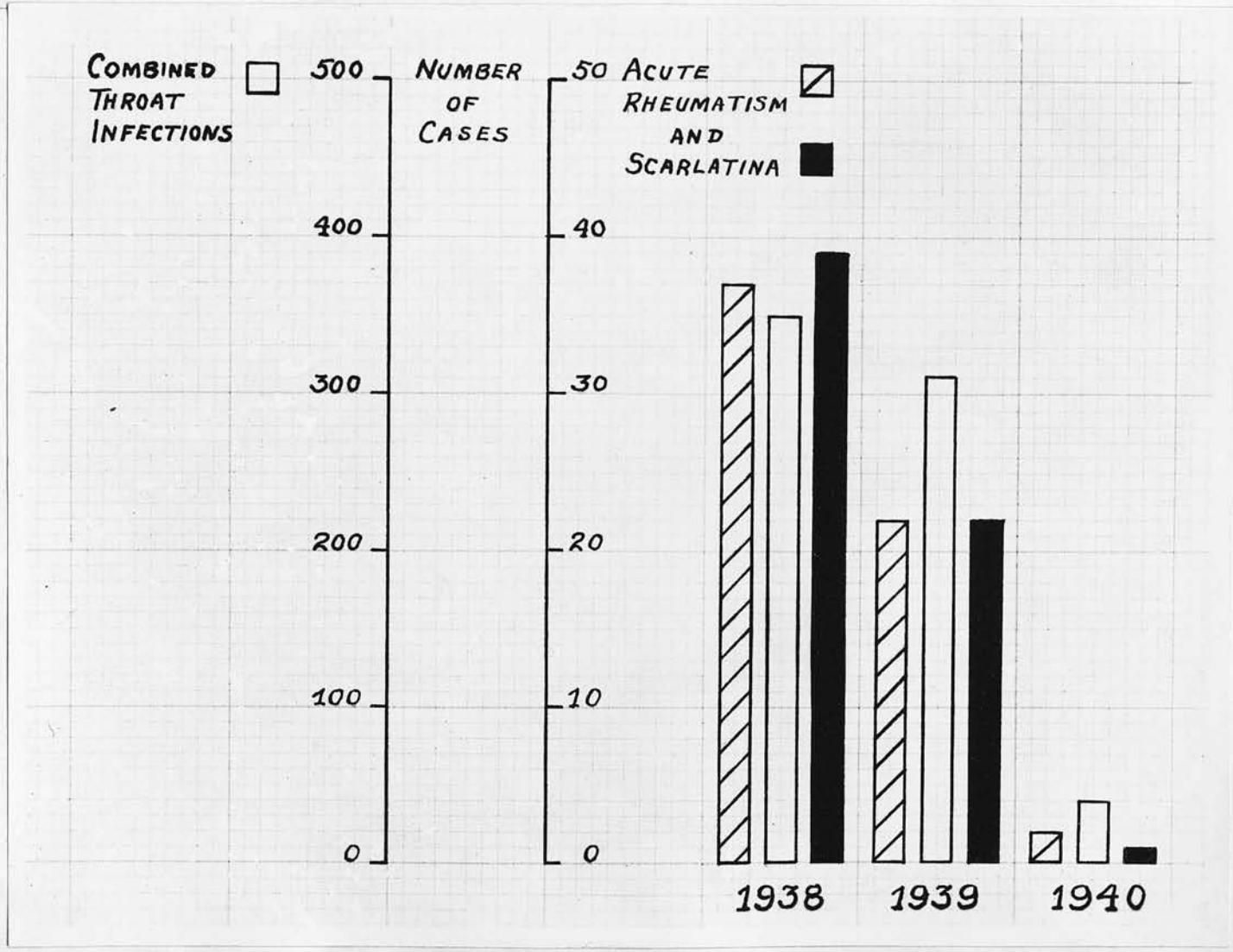
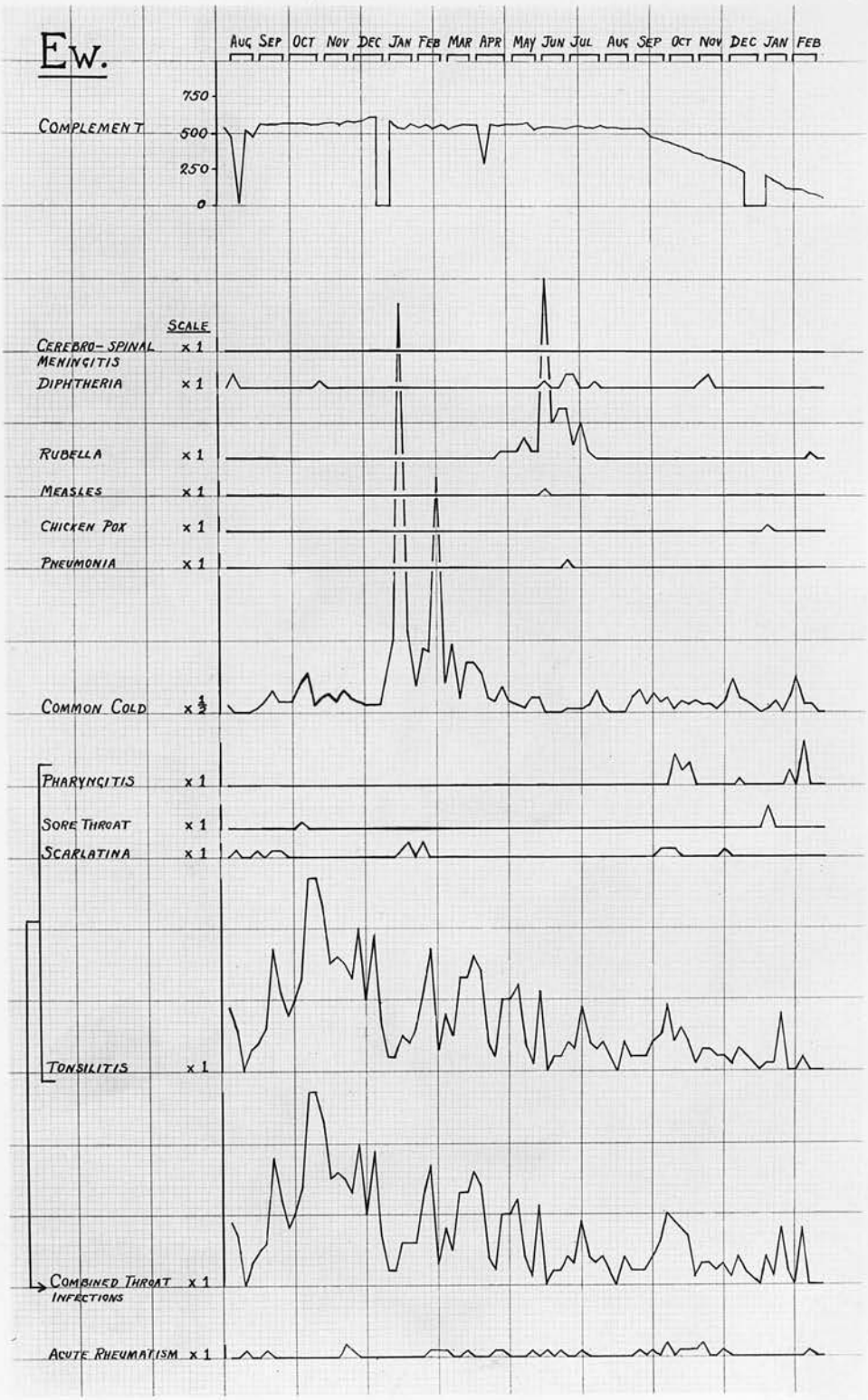


CHART 7 : TO CONTRAST THE TOTAL NUMBER OF CASES OF COMBINED THROAT INFECTIONS, SCARLATINA AND ACUTE RHEUMATISM IN TRAINING CENTRE Sg OVER SIMILAR PERIODS IN 1938, 1939 and 1940.

CHART 8

TO SHOW THE INCIDENCE OF VARIOUS INFECTIONS AND OF ACUTE RHEUMATISM IN TRAINING CENTRE Ew.



aged fifteen to seventeen years, and were joined in small groups at short intervals.

Epidemics were experienced as follows:-

Disease	Term	Year
Rubella	Summer	1939
Common Cold	Spring	1939

In the past, acute rheumatism has been prevalent at this centre. In 1939, twenty cases were notified, giving an incidence for the year of 36 per 1000.

Tonsillitis was common in every term and the estimated incidence of combined throat infections in 1939 was 450 per 1000. Despite the prevalence of throat infections, only 9 cases of scarlatina were notified in 1939, giving an estimated incidence per 1000 of 16.4. Cases of rheumatism occurred throughout the whole period.

DISCUSSION

The investigation has indicated the complete absence of any relationship between acute rheumatism and epidemics of rubella, measles, chicken pox, common cold and diphtheria. On the other hand, the distribution of rheumatic cases was similar to that of tonsilitis which was mainly of haemolytic streptococcal origin. Thus centres such as Hd(p.185) or Dc(p.187) , in which rheumatism was infrequent, produced a correspondingly low incidence of throat infections, and such epidemics as did occur were mild and seasonal. When

infection was rife, as in centre Sg, the figures for rheumatism were also high. This does not mean that scarlatina, tonsilitis and acute rheumatism could be exactly correlated, and the difficulties in attempting this are illustrated in Chart 9 (p.200). The chart was constructed with data, referring to the same period of time, from four of the centres described in the text, together with the figures from the institution Ac, which had been reported in section I, paper 2. It will be seen that two of the centres, Sg and Ew, had the same rheumatism rate of 22 per 1000, but the throat infection rates per 1000 differed markedly, being 148 and 778 respectively. The incidence of scarlatina, however, was slightly higher in centre Sg. As the exposed populations were of the same age-group, the different rates for combined infections and for scarlatina probably indicated that streptococcal infection in Ew, though more widespread, was milder in type. As another example of difficulty in correlation, it may be seen that the rheumatism rate in Ac was thrice that of Ew, whereas the throat infection rate was less than half. Here again the incidence of scarlatina was proportionately high.

The effect of age on the incidence of rheumatism in training centres was well demonstrated in the centre Sg. The experimental conditions were ideal except for the fact that the two groups were, of necessity, in occupation at different times. The

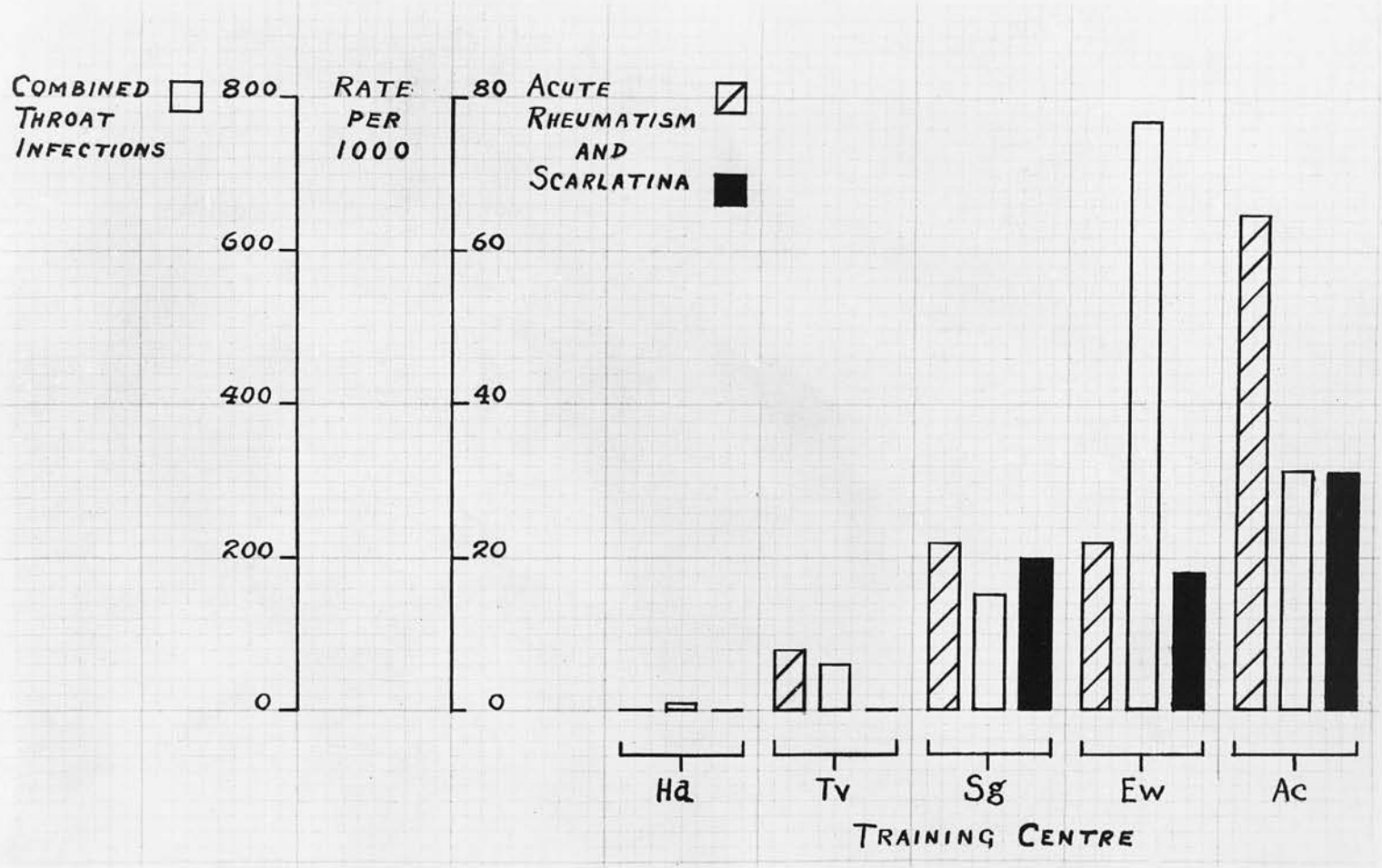


CHART 9 : TO COMPARE THE INCIDENCE OF COMBINED THROAT INFECTIONS, SCARLATINA AND ACUTE RHEUMATISM IN FIVE TRAINING CENTRES DURING THE SAME PERIOD OF TIME.

climatic conditions were similar in each year and could not be used to explain the difference in incidence of rheumatism. The evidence indicated that the more frequent occurrence of acute rheumatism during the winter months was due to the simultaneous increase in respiratory infections and was not the result of climate per se. If the usual conditions are reversed and infection becomes more common in the summer, then rheumatism is also more in evidence. This has been demonstrated already in training centre Ac (section I, paper 2), when rheumatism reached epidemic proportions during a widespread outbreak of tonsilitis and scarlatina during the summer term of 1938.

The importance of age was also shown in the comparative absence of rheumatism in the centre Dc, despite the sixfold increase in personnel during the first year of its existence. Provided the individual resistance to streptococcal infection is high and epidemics are absent, then acute rheumatism ceases to be a danger in such training centres or similar communities.

SUMMARY

The distribution of various infectious diseases in certain training centres is discussed in relation to the streptococcal aetiology of acute rheumatism. The relative importance of the age of trainees and environment are also discussed.

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SECTION J

PRELIMINARY OBSERVATIONS ON THE USE OF CONVALESCENT SERUM IN THE TREATMENT OF ACUTE RHEUMATISM

INTRODUCTION

In certain communities of young, male adults engaged in strenuous physical training, there has been a considerable amount of acute rheumatism. In a few individuals there was a definite history of some manifestation of rheumatism before admission to the training establishment, but in many the first attack was experienced within a few weeks of entry. The mode of onset was extremely varied. In the majority of cases nasopharyngeal infection antedated the insidious onset of stiffness and pain in one or more joints which demanded medical attention, and the patient was then found to be febrile. In others, the disease appeared with dramatic onset of hyperpyrexia, sweating and polyarthritis, as in typical rheumatic fever. On the whole, the joint lesions in this latter group of cases have not been striking. Large effusions were not

common, but tenderness, slight puffiness and oedema of surrounding tissues, were the usual manifestations. Flitting in character, the joint lesions rapidly improved and left no apparent injury of permanent character. The initial pyrexia rarely lasted longer than one week irrespective of the treatment aopted, but exceptional cases continued febrile for months. Remissions and relapses occurred frequently and symptoms were greatly relieved by salicylate therapy. Despite the apparent mildness of infection in the early stages of the disease, the cardiac complications have been all too frequent, and have occasioned great anxiety on account of the resulting incapacity. Fortunately, a number of patients did not show any tendency to develop cardiac lesions. As these were otherwise healthy young adults, it was decided to try the effect of serum, taken from such patients during convalescence, in the more acute phases of the disease in subsequent cases.

METHODS

Preparation of serum

Patients who were in good gemeral condition about the fourth to eighth week after the temperature had settled were selected for the supply of serum. Other criteria of suitability included the absence of all indications of cardiac complications, and the approach of the erythrocyte sedimentation rate to

normal limits. From each patient, 300 c.c. to 400 c.c. blood were obtained, the serum separated, filtered, and prepared by the addition of 0.3 per cent phenol. Inthis preliminary investigation, the serum from each patient was subjected to the Wassermann reaction and, if negative, was ampouled. Routine sterility tests were performed on each batch of serum.

Dosage

The serum was given either intramuscularly or intravenously in doses of 10 to 20 c.c. Exceptionally larger doses were used as detailed in individual cases.

Additional therapy

As far as possible, no form of treatment other than general nursing and local therapy were used in conjunction with serum. Depending on the preference of the clinician in charge of the case, additional measures such as the use of salicylates, supplemented serum therapy.

RESULTS

Serum has now been used in the treatment of 15 cases divided into two groups; those treated by serum alone, and the remainder in whom serum therapy supplemented the action of other modes of treatment such as salicylates.

Serum without salicylates

In 10 cases, no salicylates or allied preparations were used throughout the illness. Of these,

seven cases, which included six primary attacks and one recurrence, reacted favourably to the treatment. Details of two of these successfully treated cases were as follows:-

Case 1.- G.M. Mx.57749 , aged 15 years.

Family History.- Mother,father and two siblings- alive and well with no history re rheumatism.

Previous history.- Tonsillectomy at 8 years-no rheumatism.

Present history.-

21.8.38: Joined training establishment.

8.11.38: Common cold.

30.11.38: Chart 1(p.208). Admitted to hospital, complaining of joint pains in arms and legs. The knee-joints were both swollen, extremely painful and, in the right knee, there was a considerable effusion. The condition remained unchanged for next five days in absence of any therapy other than careful nursing and local treatment.

6.1.39 to 9.1.39: Intramuscular injection of 10 c.c. convalescent serum given daily. The pains in the joints were greatly eased within a few hours of the first injection, and the patient passed his first good night on the sixth day of illness. There was no further joint lesion after the ninth day, and the temperature had returned to normal within six days of serum being first given. A mitral systolic murmur was noted on the fifth day of disease but disappeared

during the fifth week. Apart from marked bradycardia during the second week, the remainder of convalescence was uneventful. There was no relapse, no evidence of valvular disease on discharge, and the patient has been on duty for several months in apparent good health.

Case 2.- K.A. JX. 162936 , aged 15 years.

Family History.- Nothing relevant.

Previous History.- No rheumatism- tonsilitis infrequent.

Present History.- Chart 2 (p. 208).

29.8.39: admitted to hospital with complaint of pain and swelling of left ankle and, later, right ankle. The right knee was definitely swollen, very tender, and both ankle-joints were stiff and sore. Condition remained unchanged on following two days.

31.8.39 to 2.9.39: Intramuscular injection of 10c.c. convalescent serum given daily. The temperature and pulse-rate progressively fell during next four days and pain was relieved within twenty-four hours. Convalescence was uneventful without any relapse. Finally returned to duty without evidence of cardiac damage.

The five other cases were of this same type: convalescent serum, given in the early stage of the disease, was followed by a rapid fall in temperature, and speedy relief of the symptoms. Six of the seven successfully treated cases were primary attacks, and the seventh was a recurrence. No case has relapsed since treatment and all have returned to duty.

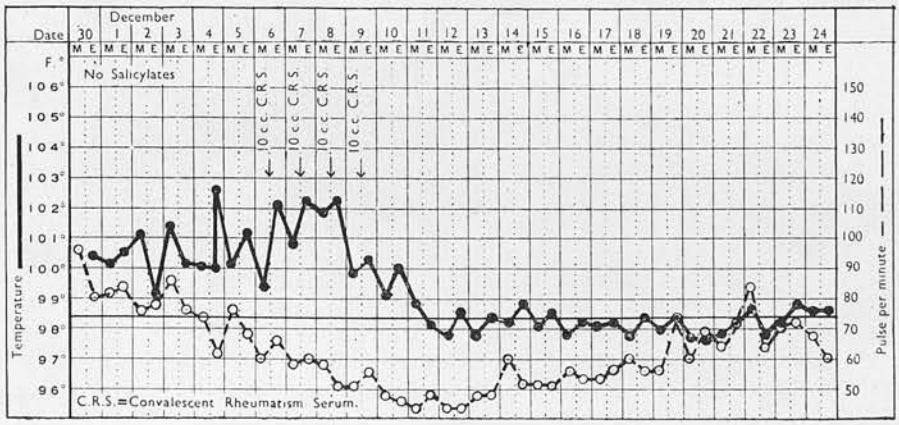

CHART 1 CASE 1

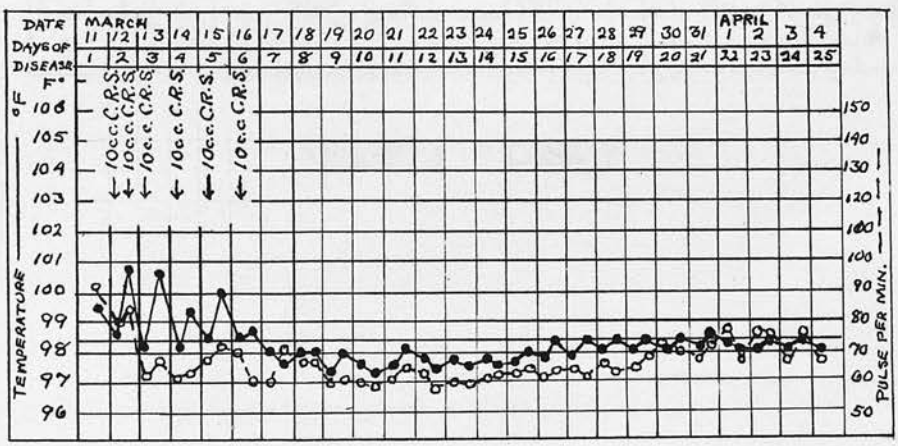

CHART 3 CASE 3

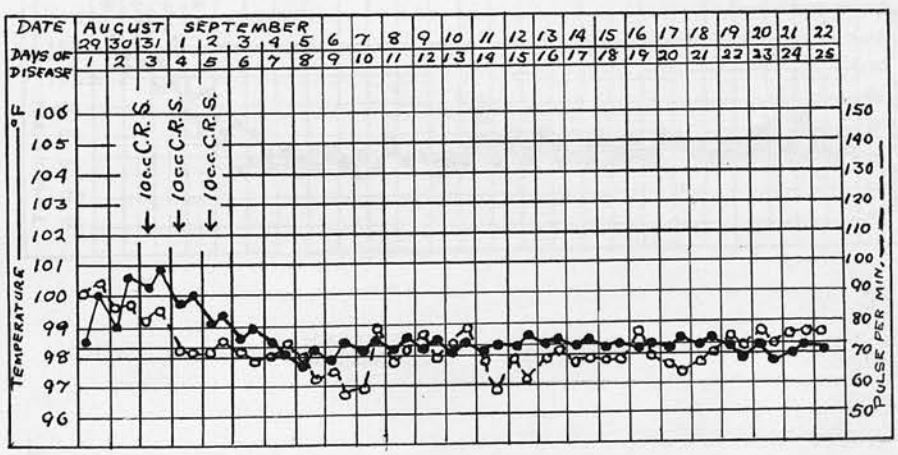

CHART 2 CASE 2

SECTION J CHARTS 4 and 5

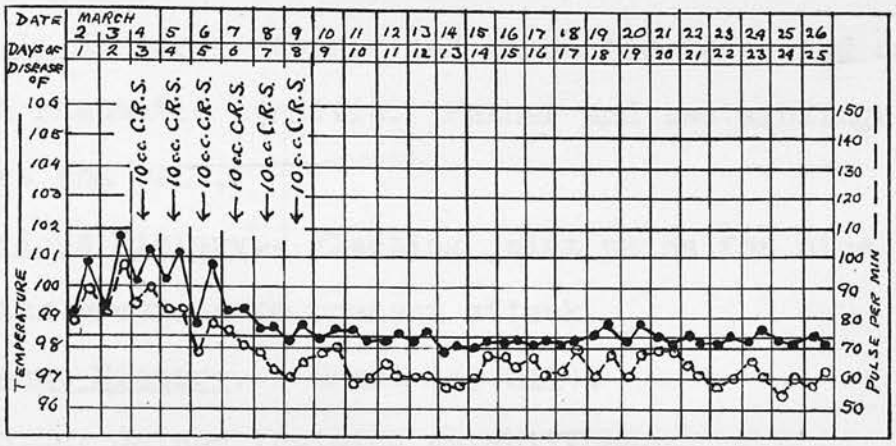

CHART 4 CASE 5

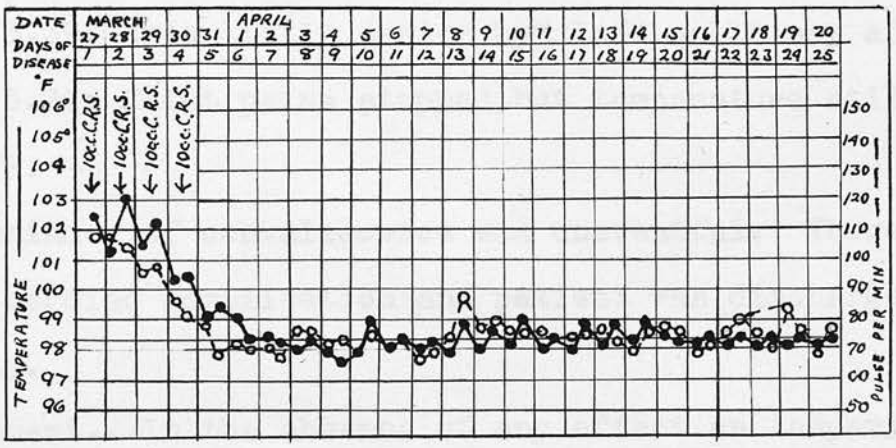

CHART 5 CASE 6

Details of the three cases of this group which did not react favourably to serum therapy were as follows:-

Case 8.- W.E. 3148/39 , aged 27 years.

Family History.- Mother had rheumatic fever and died from rheumatic carditis. Father and two siblings alive and well.

Previous History.- Fleeting joint pains for nine months previous to present attack.

Present History.- Chart 6 (p.211).

20.3.39: Joined training establishment.

9.5.39: Admitted to hospital complaining of pain in both knees. The left knee was definitely swollen, warm and very tender.. On the same day, 20 c.c. convalescent serum were given intramuscularly.

10.5.39: Knee-joint still swollen and temperature rising.

11.5.39: Knee little easier but left ankle now affected.

12.5.39: Joint pains stopped but temperature still 99°F.

Remainder of convalescence was uneventful. There was no cardiac complication and patient was discharged to duty.

Comment.- In the absence of any effect on temperature and on account of extension to left ankle-joint, serum had no effect in this case. Only one dose was given

SECTION J CHART 6

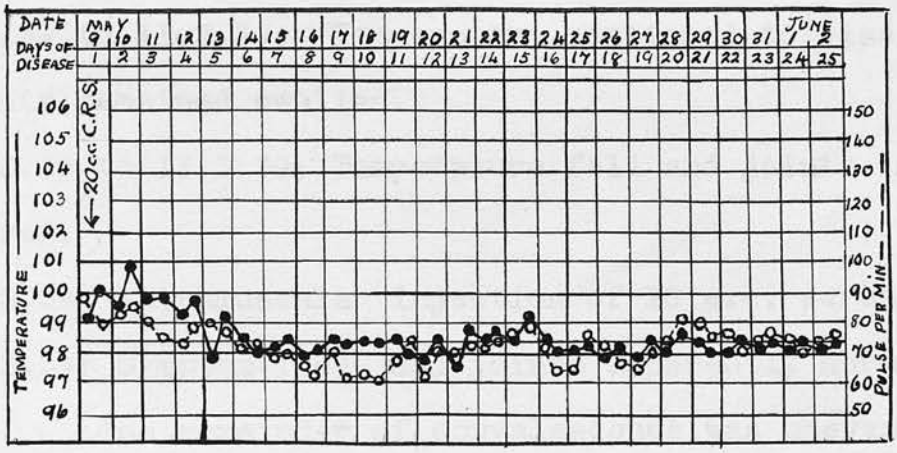

CHART 6 CASE 8

and continued treatment might have been more sucessful.

Case 9.- A.M. 3136/39 , aged 18 years.

Family History.- Nothing relevent to rheumatic disease.

Previous History.- No previous history of disease.

Present History.- Chart 7 (p.213).

8.3.39: Admitted with complaint of pain in soles of feet and later in knees. Pain, swelling, and tenderness of both wrists. 20 c.c. convalescent serum given intramuscularly.

9.3.39 to 11.3.39:- Temperature continued to rise, and joints remained swollen.

12.3.39 to 13.3.39: Temperature fell and joints improved.

14.3.39: Intramuscular injection of 10 c.c. serum.

15.3.39: Symptom-free, and joints apparently normal.

The remainder of convalescence was uneventful, despite the slow return to normal limits of the erythrocyte sedimentation rate and formol-gel reaction. There were no cardiac complications and the patient returned to duty.

Comment.- The first injection of serum had no effect in arresting the disease, and although the second injection was followed by amelioration of symptoms, the clinical chart indicated that this was a chance association. However, it was noted that there were no further joint manifestations and no cardiac lesions,

SECTION J CHART 7

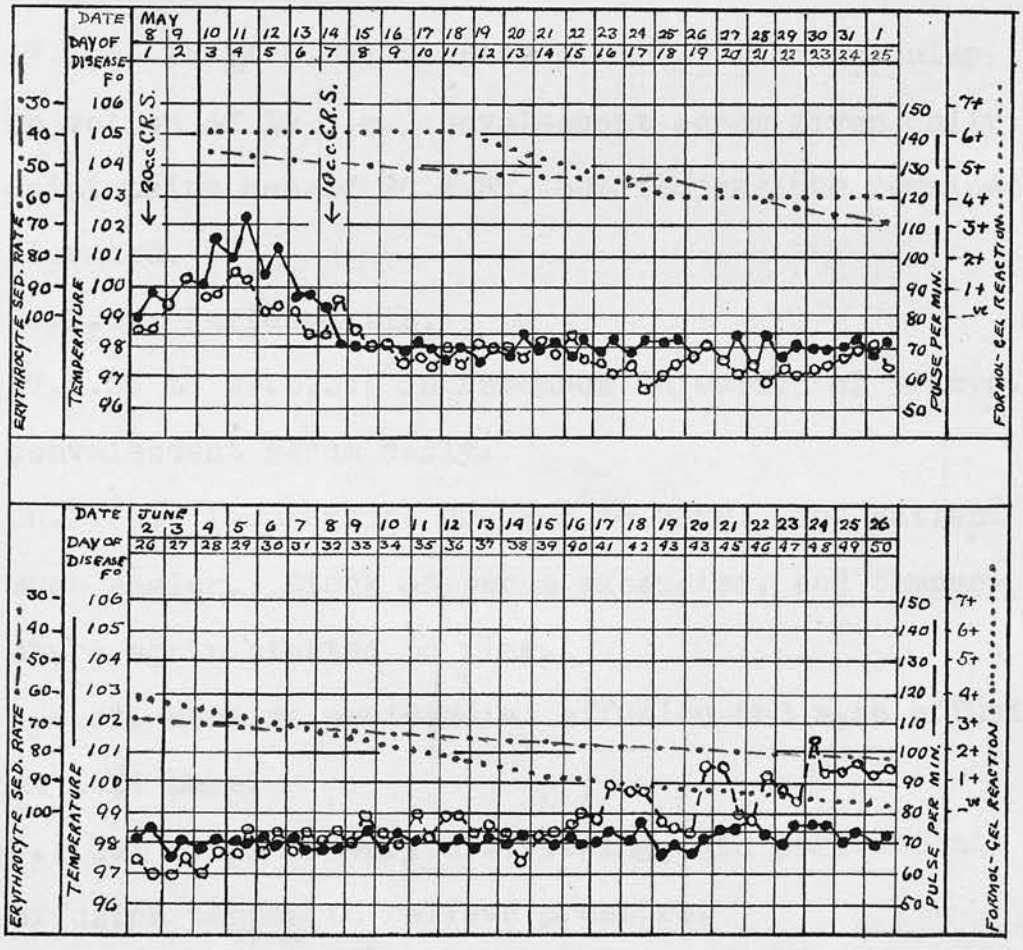

CHART 7 CASE 9

although the sedimentation rate remained abnormal for a further three weeks.

Case 10.- A.M. 1840/ F.T. , aged 16 years.

Family History.- Nothing relevant to rheumatism.

Previous History.- Measles, rubella, no rheumatic manifestations.

Present History.-

10.1.39: Joined training establishment.

14.2.39: Common cold and tonsilitis.

14.3.39: Complained of generalised joint pains and abdominal pain.

17.3.39 to 21.3.39: Chart 8 p.215 . Intramuscular injection of 10 c.c. convalescent serum given daily. Joint pains ceased 20.3.39, but temperature remained elevated.

24.3.39: Pericarditis.

27.3.39 to 29.3.39: Intravenous injection of 50 c.c. convalescent serum daily.

30.3.39: Temperature dropped to 99°F., and patient much easier. Stock of serum exhausted, and temperature again started to rise.

5.4.39: Massive pericardial effusion and also effusion at left base.

6.4.39: Dyspnoea very distressing, and pericardial effusion tapped to relieve pressure.

7.4.39: Condition unchanged, and tapping repeated.

11.4.39: Pleurisy at right base, and consolidation at left base, pericardial effusion decreasing.

SECTION J CHART 8

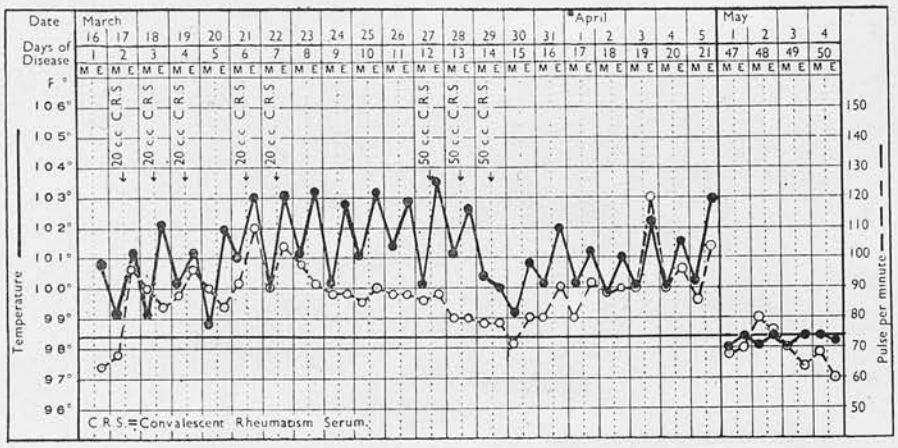

CHART 8 CASE 10

He made a steady recovery, the pneumonitis and pericarditis clearing up. A blowing, mitral systolic murmur was heard when the pericardial rub ceased, but this finally disappeared, and he was discharged to duty looking extremely fit. He has now been on duty without relapse.

Two blood cultures during the pyrexial period and both pericardial and pleural effusions were sterile.

Comment.- Despite the failure of the initial course of serum to prevent the onset of pericarditis, the clinician was of the opinion that the large doses of the second course had a beneficial effect on the complication. Unfortunately, the stock of serum was exhausted. The temperature chart also suggested that the process was temporarily arrested by the serum therapy.

In this group of ten cases, nine have now been at duty for several months without relapse, and with no evidence of permanent cardiac damage. The remaining case probably has a valvular lesion, but tolerance has always been good and he is also back on duty.

Serum and Salicylate Therapy

In five cases serum was used during some stage of treatment in combination with or as an alternative to salicylates.

Case 11.- L.W.B. 550788. 6386 , aged 19 years.

Family History.- Nothing relevant.

Previous History .- Rheumatism three years previously.

Present History.- Chart 9(p.218)

Started with pain in both feet. Next day, both knees were affected, and on the following day, both wrists.

5.12.38: Both wrists, right knee and right foot, swollen, tender, and painful. Pain also in both shoulders and back of neck. Convalescent serum not then available, and salicylate therapy was started. Response satisfactory.

13.12.38: Left wrist only slightly painful. Temperature continued in normal limits from 8.12.38 and the erythrocyte sedimantation rate and formol-gel reaction settled. Salicylates stopped 31.12.38.

5.1.39: Left wrist again swollen and painful. Salicylates again checked temperature. Convalescence was uninterrupted for next thirty days, but on 16.2.39 sedimentation rate and formol-gel reaction rose for no apparent reason.

24.2.39: Relapse with pyrexia (T.100.1°F.), pain, swelling and tenderness of right elbow, right wrist, and left knee. 10 c.c. convalescent serum given intramuscularly.

27.2.39: Temperature normal.

28.2.39: Salicylates restarted.

SECTION J CHART 9

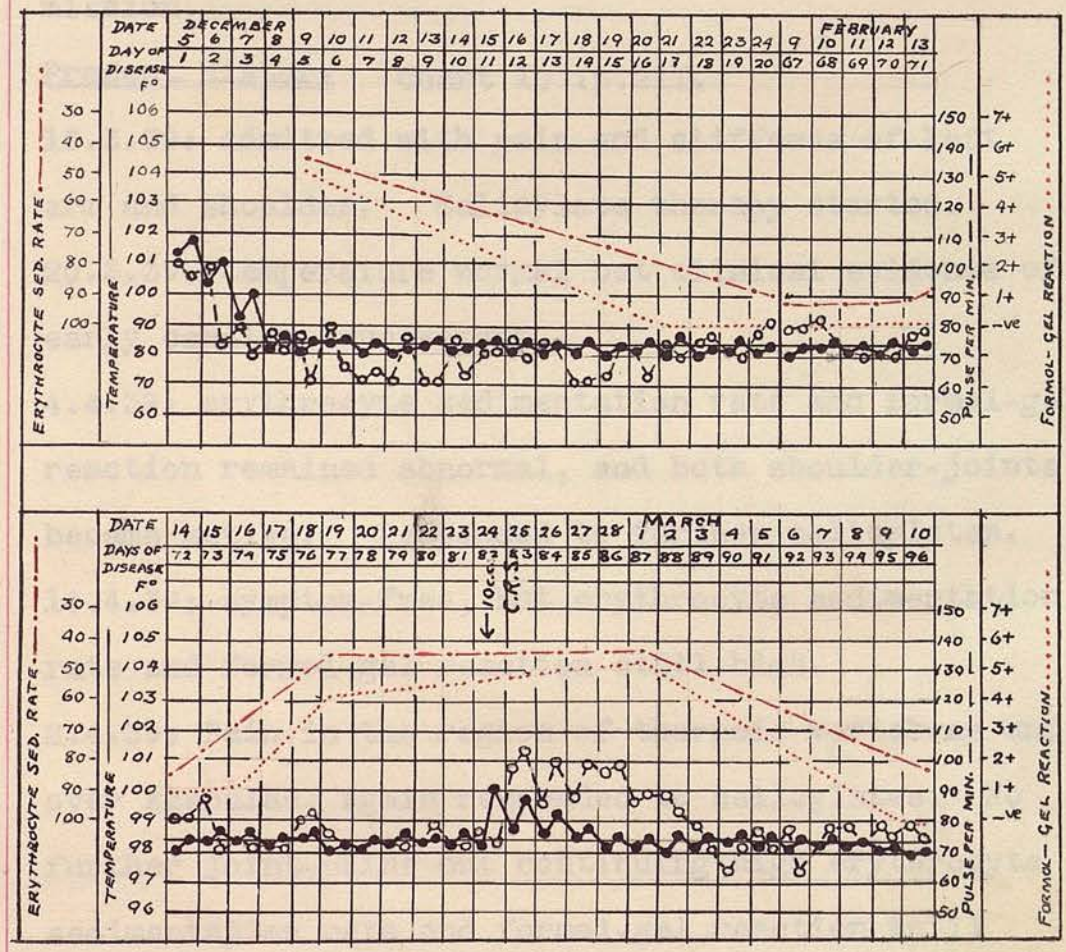

CHART 9 CASE 11

Convalescence continued uneventful with no evidence of carditis at any stage, and he was discharged for duty.

Comment.- The actual effect of serum on this relapse was obscured by the use of salicylate. However, the temperature had returned to normal and the pains were relieved before salicylate therapy was restarted.

Case 12.- F.W. JX. 161128 , aged 15 years.

Family History Nothing relevant.

Previous History Tonsilitis six weeks prior to admission.

Present History Chart 10 (p.211).

16.3.39: admitted with pain and stiffness of left arm and shoulder. Salicylate therapy started.

20.3.39: temperature normal but clinical evidence of early cardiac involvement.

4.4.39: erythrocyte sedimentation rate and formol-gel reaction remained abnormal, and both shoulder-joints became active. Responded to further salicylates.

10.4.39: symptom-free, but erythrocyte sedimentation rate and formol-gel reaction still high.

2.4.39: Pain in the region of thoracic vertebrae and over scapulae, again responded to salicylates. No further joint-pains but continuing high erythrocyte sedimentation rate and formol-gel reaction until 3.6.39: right wrist joint: 4.6.39: right elbow-joint.

1.7.39: digitalis on appearance of auricular fibrillation.

5.8.39: complaint of pericardial pain and rapid rise in temperature and pulse-rate heralded onset of pericarditis. No salicylates were given at this time, but after six days 10.8.39 15 c.c. convalescent serum were given intramuscalarly.

11.8.39: slight fall intemperature. Intravenous injection of 10 c.c. convalescent serum.

12.8.39: general condition improved, pulse stronger and more regular. Intravenous injection of 20 c.c. convalescent serum.

13.8.39:improvement maintained.

14.8.39: 20 c.c. convalescent serum intravenously.

20.8.39 : 20 c.c. convalescent serum intravenously. Within two minutes marked dyspnoea and cyanosis, pulse almost imperceptible. Adrenaline and oxygen given: patient rallied in few minutes.

21.8.39: felt much improved.

23.8.39: 20 c.c. convalescent serum intravenously. Again respiratory distress within five minutes, relieved by adrenaline and oxygen in thirty minutes.

24.8.39 to 7.9.39 : uneventful.

Comment.- This case indicated the gravity of the disease and left no doubt as to the diagnosis. As shown, salicylate therapy alleviated the joint pains but did not prevent the recurrence of arthritic lesions, nor the development of pericarditis. It was interesting to note the continued abnormal erythrocyte sedimentation

SECTION J CHART 10

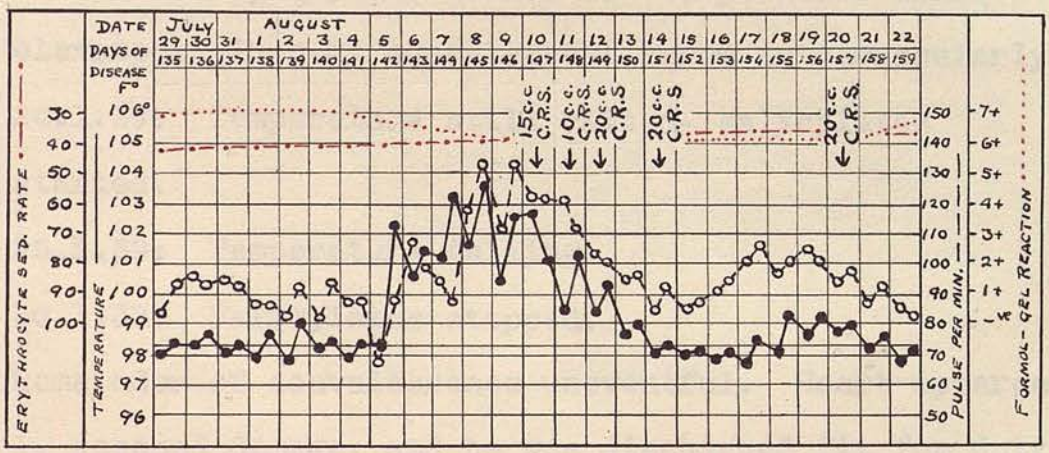

CHART 10 CASE 12

rate and formol-gel reaction, despite the apparent clinical inactivity of inrection over long periods. A disturbing feature of this case was the collapse after the fifth and sixth serum injections.

Case 13.- J.E.B. MX. 57652. 485/39 , aged 16 years.

Family History.- Nothing relevant to rheumatism.

Previous History.- Tonsilitis infrequent - no rheumatism.

Present History.- Chart 11 (p.223).

19.1.39: Effusions in both knee-joints, particularly the left. Elbow-joints tender and painful.

20.1.39: 10 c.c. convalescent serum intramuscularly.

21.1.39: Joint pains ceased but temperature still elevated. 10 c.c. convalescent serum intramuscularly.

24.1.39: Temperature still raised- salicylates started.

25.1.39: Temperature falling.

30.1.39: Salicylates stopped.

Remainder of convalescence uneventful. Heart apparently escaped damage, and he was discharged fit for duty.

Comment.- Although possibly contributing to the relief of pain, serum had no effect on temperature nor onn the general condition. Only a small dosage was used.

Case 14.- T.A.M. SSX 26242. 893/39 , aged 17 years.

Family History.- Mother has had rheumatic fever. Father and two siblings alive and well.

Chart 12 (p.224).

Previous History.- No rheumatism- tonsilitis infrequent, but last attack within six weeks of admission.

SECTION J CHART 11

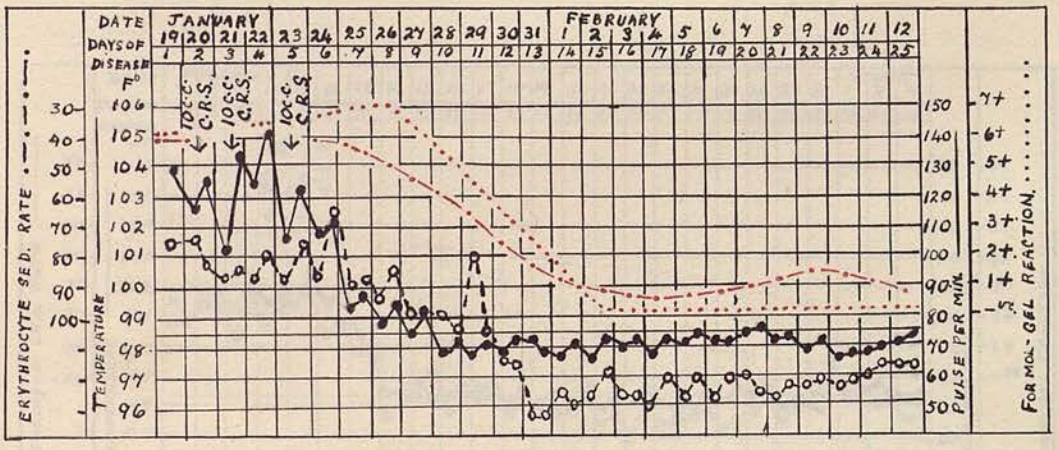

CHART 11 CASE 13

SECTION J CHART 12

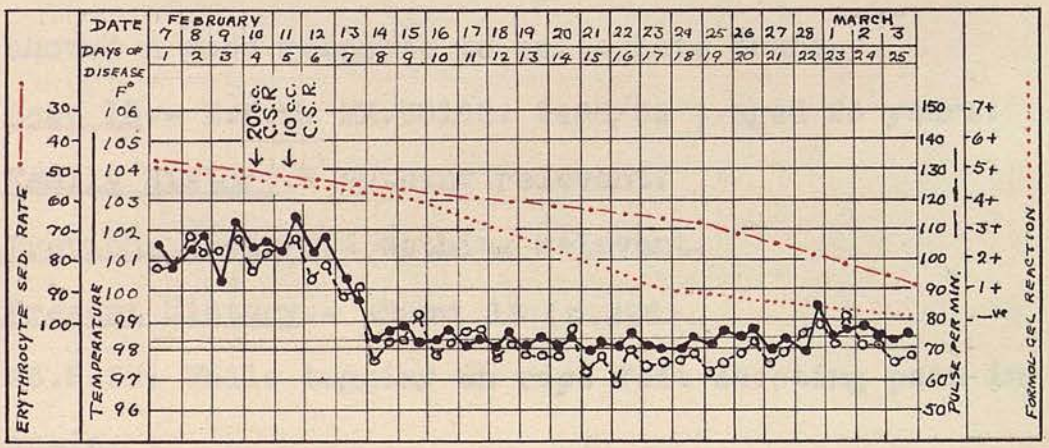

CHART 12 CASE 14

Present History.- Complained of aching in all joints.

10.2.39: Admitted with both knees and ankle-joints swollen and tender. Intramuscular injection 10c.c. convalescent serum, repeated in afternoon.

11.2.39: Pains easier but still pyrexial. Intramuscular injection of 10c.c. convalescent serum.

13.2.39: Condition unchanged and salicylates started. Good response to salicylates.

Remainder of convalescence uneventful, with no evidence of carditis.

Comment.- Serum had no effect in this case, which showed a good response to salicylate therapy.

Case 15.- E.P.M. MX.58162. 3486/39 , aged 26 years.

Family History.- Nothing relevant.

Previous History.- Nothing relevant.

Present History.- Chart 13 (p.226).

25.5.39: While tugging on rope felt shooting pain in groin.

26.5.39: Both ankles stiff and sore.

27.5.39: Admitted to hospital with knees and ankles swollen and very painful- salicylates started.

5.6.39: Both knees affected- salicylates restarted. Good response but abnormal erythrocyte sedimentation rate and formol-gel reaction.

20.6.39: Left knee swollen and tender-colsulanyde given.

24.6.39: Both knees again active-intramuscular injection of 20 c.c. convalescent serum- pains eased and

Present History.- Complained of aching in all joints.

10.2.39: Admitted with both knees and ankle-joints swollen and tender. Intramuscular injection 10c.c. convalescent serum, repeated in afternoon.

11.2.39: Pains easier but still pyrexial. Intramuscular injection of 10c.c. convalescent serum.

13.2.39: Condition unchanged and salicylates started. Good response to salicylates.

Remainder of convalescence uneventful, with no evidence of carditis.

Comment.- Serum had no effect in this case, which showed a good response to salicylate therapy.

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Family History.- Nothing relevant.

Previous History.- Nothing relevant.

Present History.- Chart 13 (p.226).

25.5.39: While tugging on rope felt shooting pain in groin.

26.5.39: Both ankles stiff and sore.

27.5.39: Admitted to hospital with knees and ankles swollen and very painful- salicylates started.

5.6.39: Both knees affected- salicylates restarted. Good response but abnormal erythrocyte sedimentation rate and formol-gel reaction.

20.6.39: Left knee swollen and tender-colsulanyde given.

24.6.39: Both knees again active-intramuscular injection of 20 c.c. convalescent serum- pains eased and

SECTION J CHART 13

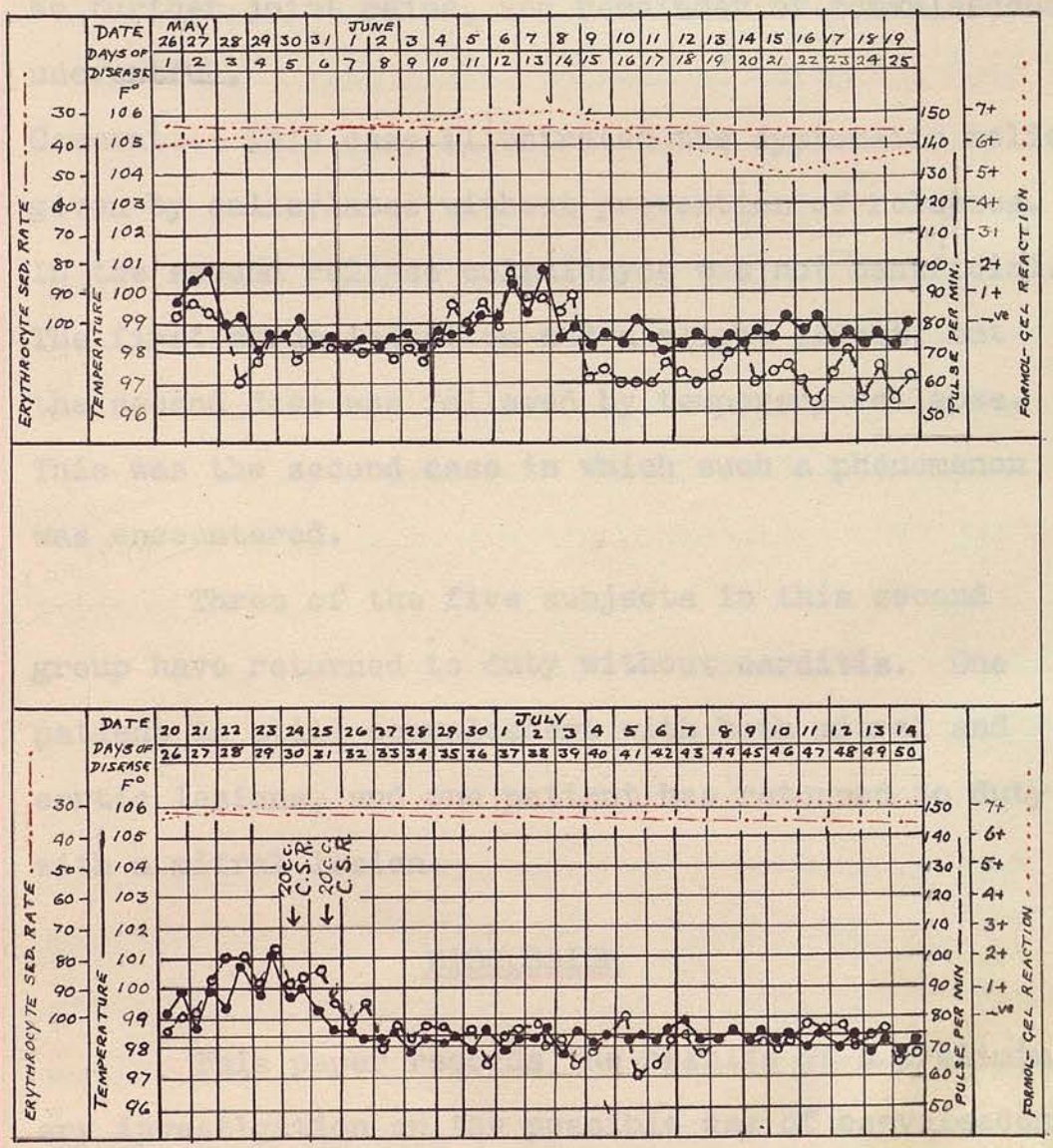

CHART 13 CASE 15

temperature remained at same level, i.e. no afternoon rise.

25.6.39: Shoulders and arms painful. 10.10 a.m. 20c.c. convalescent serum given intramuscularly; 11.20 a.m. rigor and collapse with cyanosis, shallow respirations and feeble pulse. Adrenaline and coramine; 11.35 a.m. greatly improved and 11.55 a.m. no further distress; 1 p.m. sweating profusely- no further trouble.

26.6.39: Slight pains in both shoulders but improved. No further joint pains, and remainder of convalescence uneventful.

Comment.- This case illustrated the syptomatic relief given by salicylates without prevention of relapses. In the second relapse colsulanyde was not beneficial. The first serum injection had a slight effect, but the second dose was followed by temporary collapse. This was the second case in which such a phenomenon was encountered.

Three of the five subjects in this second group have returned to duty without carditis. One patient is still convalescent with both mitral and aortic lesions, and one patient has returned to duty with a mitral lesion.

DISCUSSION

This paper records the results of a preliminary investigation on the possible use of convalescent serum in the treatment of acute rheumatism. As the clinical records show, there was a tendency for the

initial pyrexia to be of short duration, and this rendered difficult any estimation of the effect of serum on an easily recognised sign. Nevertheless, when given in the early stages of an attack, serum did appear to reduce the period of pyrexia, and this was particularly noticeable in primary attacks. Clinical study of the cases has left no doubt that arthritic pain was relieved in such cases. Of the 15 cases tried in all, nine were considered to have benefited. Other antisera have been used in the treatment of rheumatism. Thus antistreptococcal serum has been tried with varying success. Wilson (1930) and Hill (1928) reported adversely on its use, while Toogood (1926), Easson and Thomson (1934), and Small (1928) were of the general opinion that serum was of value.

The volume of serum in the present investigation was not large enough to permit really adequate dosage in every case, but the results obtained justify further extension of the method on the lines suggested.

One point which will require careful investigation, was the occurrence of partial collapse in two individuals within a short period of receiving an injection of serum. The cause of this peculiar phenomenon has not been discovered. Anaphylaxis was considered, but the time relations did not support this possibility. That it was not due simply to repeated dosage has been shown by the absence of any untoward reaction in patients receiving similar courses. Nor

could any individual batch of serum be incriminated since two separate batches were concerned, and other patients treated with the same sera have not been affected. Similar reactions have been reported by Hitchcock, McEwen and Swift (1930) following the use of antistreptococcal serum and by Poynton and Schlesinger (1937). In their cases the serum was obtained from heterologous species, whereas in the present series the serum was from the homologous species. Alarming as these two incidents were at the time, they do not present a serious contra-indication as the patients rapidly responded to appropriate treatment.

It is hoped that the investigation will now be extended, and that the true value of serum therapy will be ascertained by its effect on the course of the disease and, in particular, on the prevention of cardiac lesions, when given in adequate doses in the early stages of arthritis.

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SECTION K

REACTIONS INDUCED BY INTRADERMAL INJECTION OF RHEUMATIC JOINT FLUID : NEUTRALISATION BY CONVALESCENT SERA

INTRODUCTION

Although acute rheumatism is frequently associated with streptococcal infection, the joint fluid in rheumatic arthritis is usually found to be sterile. Of the exceptional observations may be mentioned the work of Richards (1920), and of Billings, Coleman and Hibbs (1922), who reported the isolation of streptococci in cultures of joint fluid. Cecil, Nichols and Stainsby (1929), in addition to recovering attenuated haemolytic streptococci from blood cultures in a large series of cases, found the same organism in some of the joint lesions. Examination of stained smears of joint fluid shows, in the main, polymorphonuclear leucocytes in various stages of destruction. Organisms can rarely be detected, but the occasional finding of Gram-positive cocci has been reported by Graff (1936) and by Poynton and Schlesinger (1937).

Clinically, the joint pains in acute rheumatic fever are fleeting in character, and the joints are rarely left with permanent damage after the initial attack. In these respects, the arthritic lesions appear to be due to some transient toxic action, rather than the result of actual tissue invasion.

Green, Glazebrook, Thomson and Hopkins (1939) -section J- have shown that convalescent rheumatic serum had a beneficial action when given to certain cases of acute rheumatism, but was without effect in others. In successful cases there was a rapid fall in temperature and pulse-rate with relief of symptoms. The following observations were made in an attempt to find the mechanism of this action, and thereby to formulate a method for the selection of sera for therapeutic purposes.

METHODS

Removal and treatment of joint fluid

Fluid was removed with aseptic precautions from an affected joint within twenty-four hours of the onset of symptoms by means of a wide-bore needle and syringe. The fluid coagulated overnight in the ice-chest, and on the following day partial separation had occurred. After centrifugation at 3,000 r.p.m. for thirty minutes, the bulk of the supernatant fluid was removed, leaving 1 to 2 c.c. The supernatant fluid was stored at 6°C.

Sterility tests

The coagulum and remaining supernatant fluid were mixed by shaking, and portions of the coagulum were then inoculated into the following media: Horse-muscle digest broth, 10 per cent human serum broth, 10 per cent ox serum broth, 5 per cent horse blood-agar and Loeffler serum. The chorio-allantoic membranes of ten-day-old fertilised eggs were also inoculated.

Intradermal tests

Preliminary tests in rabbits and in guinea-pigs had shown that the intradermal injection of rheumatic joint fluid failed to elicit any visible skin reaction. The remaining tests were all performed in one human subject, using the separated supernatant fluid. The test subject A was a healthy male, aged 31 years, with no history of acute rheumatism. The results were read at twenty-four, fourty-eight, and seventy-two hours, and recorded as the longest diameters in mm. of the zone of erythema.

Neutralisation tests

These were performed whenever possible. Sera were obtained at frequent intervals by venous puncture during various phases of illness, and kept without preservative at 6°C. Equal volumes of joint fluid and serum were mixed, incubated at 37°C. for four hours, and the test dose, o.3 c.c. of the mixture, was then injected intradermally into subject A.

SECTION K CHART 1

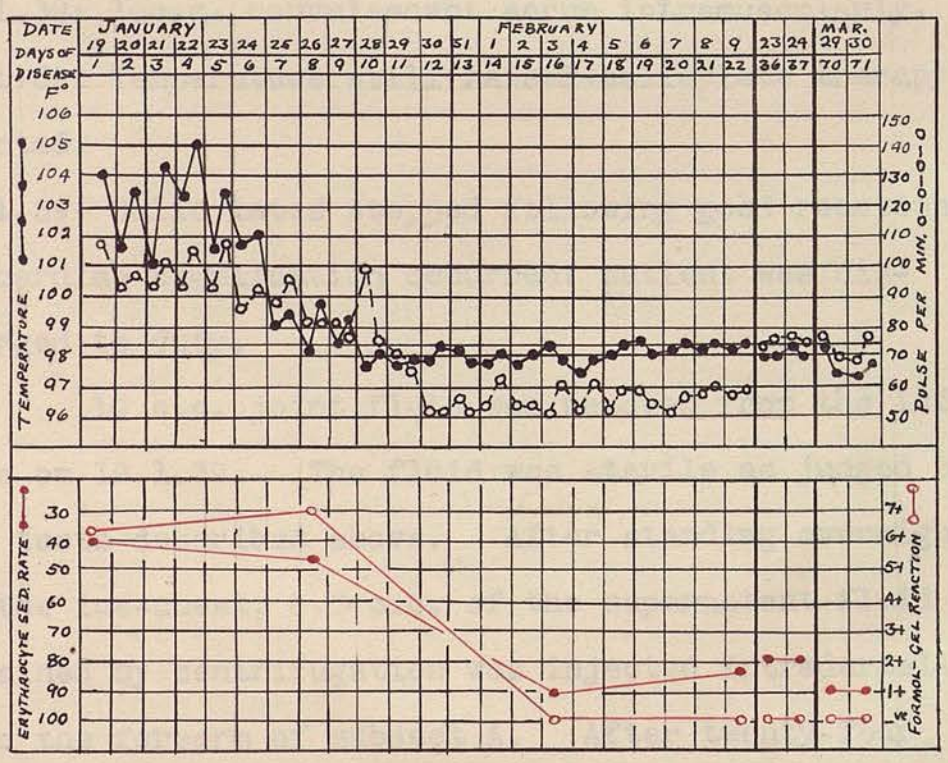

CHART 1 CASE 1

RESULTS

The observations were made in the following cases of acute rheumatic fever:-

Case 1.- J.E.B., MX 57652. 485/39. Aged 16 years.

Family History.- No rheumatism.

Previous History.- Tonsilitis infrequent- no rheumatism.

Present History.- Chart 1(p.232)

19.1.39: Acute pain and swelling in both knee-joints, and elbow joints.

20.1.39: 10c.c. convalescent serum intramuscularly.

24.1.39: Temperature still raised-salicylate therapy started.

30.1.39: Salicylates stopped following good reaction. No cardiac complication occurred: patient was discharged to duty.

10 c.c. joint fluid was removed from the left knee on 19.1.39. The fluid was sterile as judged by the tests described above. After standing overnight in the ice-chest, 0.3 c.c. of the supernatant fluid obtained by centrifugation was injected intradermally into the forearm of subject A. After twenty-four hours the site of injection was surrounded by an area of erythema 40 X 80 mm. The centre of the reaction was brighter than the surrounding zone, the edge being quite distinct. After forty-eight hours, the area had increased to a maximum of 120 X 60 mm. and was tender and swollen. The bright coloration subsequently faded and the swelling decreased, but at the centre

a small papule persisted for several days.

Specimens of sera were taken from the case J.E.B. during the first ten weeks of illness, and the effect of admixture of such sera on the capacity of the joint fluid to produce an erythematous reaction in subject A was determined. The results are given in Table 1 (p.239). It was noted that as a result of storage and incubation at 37°C. the activity of the joint fluid had deteriorated. The zone of erythema at forty-eight hours resulting from the injection of 0.3 c.c. of the stored fluid was 30 X 50 mm. (col.6), as compared with 60 X 120 mm. in the case of the fresh fluid. The serum control (col.7) indicated that the serum of patient J.E.B. was ineffective in subject A. Sera taken during the convalescence of the patient J.E.B. (col. 2,3 and 4) had a definite neutralising action in preventing the appearance of the erythematous reaction. On the other hand, serum taken on the day that knee-joint fluid was obtained, failed to neutralise (col.1), although the zone of erythema was reduced as compared with the test dose of joint fluid (col.5). The clinical data and sedimentation rates showed that the patient was acutely ill on 19.1.39, but was greatly improved on the days on which subsequent specimens of serum were tested.

Case 2.- K.B., 553126, aged 16 years.

Family History.- Nil relevant.

SECTION K CHART 2

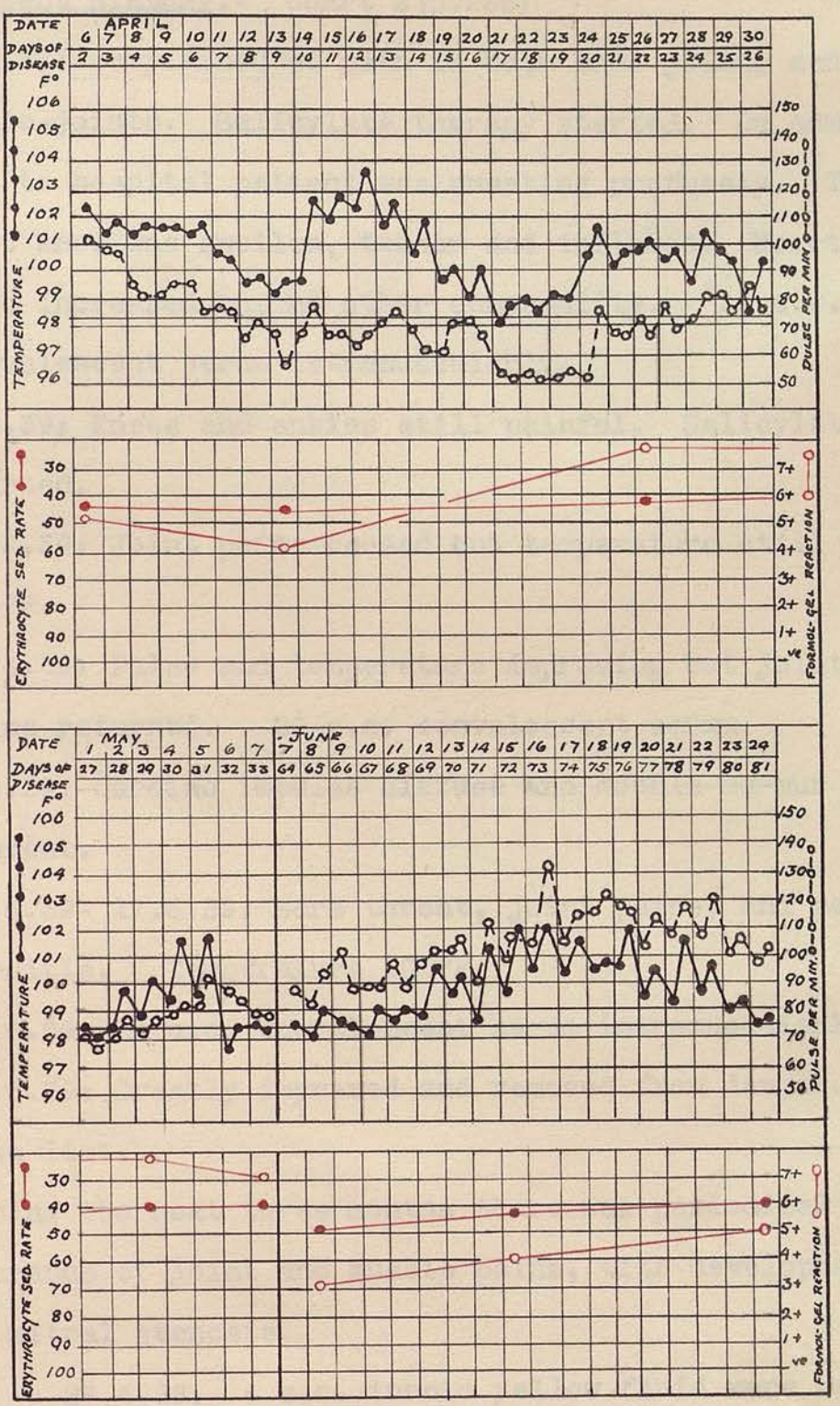

CHART 2 CASE 2

Previous History.- Nil relevant.

Present History.- Chart 2 (p.235)

5.4.39: Complained of pain in both knee-joints and ankle-joints. Salicylate therapy started. On admission to hospital patient was sweating profusely. The left knee was swollen, tender and inflamed. Heart-rate increased but no other abnormality. 20 c.c. convalescent serum intramuscularly.

8.4.39: Knees and ankles still painful. Salicylates started.

10.4.39: Joint pains ceased but temperature still 100°F.

14.4.39: Pulse and temperature improving but joint pains returned. 20 c.c. convalescent serum.

5.5.39: Cardiac impulse diffuse and double murmur now present.

15.6.39- 17.6.39: Sore throat, joint pains, and pericarditis. Temperature 103°F.

22.6.39: 20 c.c. convalescent serum intramuscularly.

24.6.39: Greatly improved and removed from dangerous case list.

During the next three months there was periodical appearance of joint and muscle pains, with development of mitral stenosis.

27.4.39: 4 c.c. turbid yellow fluid were removed from the left ankle-joint within twenty-four hours of the onset of pain and swelling. Culture tests were all negative. The intradermal injection

SECTION J CHART 3

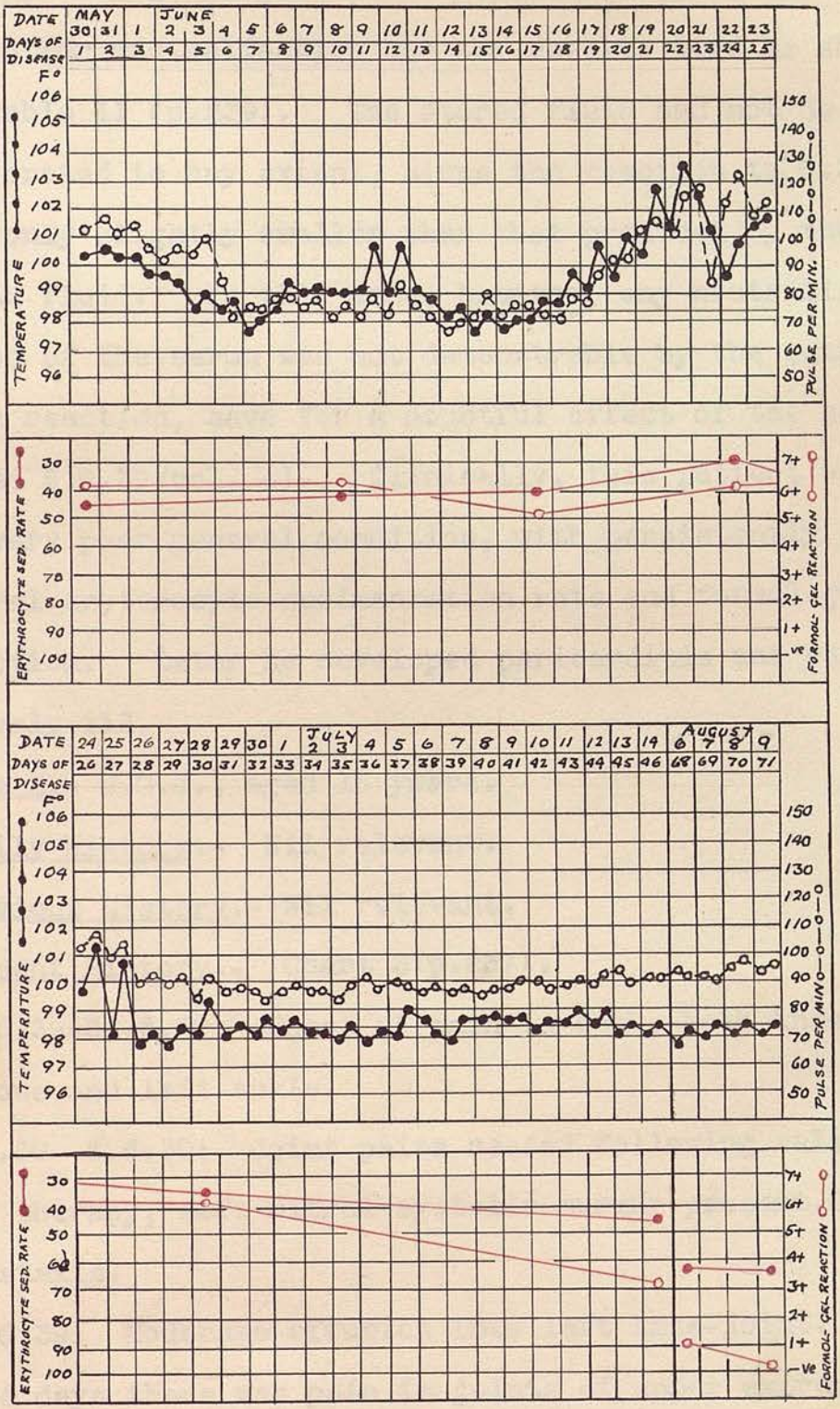

CHART 3 CASE 3

of o.3 c.c. of the fresh fluid produced a reaction of 40 X 60 mm. in subject A. Specimens of sera were taken for neutralisation and the results were as shown in Table II (p.239). The stored fluid had not deteriorated to any extent, since the reaction to 0.3 c.c. was only slightly smaller than that produced by the fresh fluid. In this case, however, any neutralisation of the serum was not demonstrable by the cutaneous reaction, save for a doubtful effect of the serum dated 8.6.39 (col. 4). Clinically, this patient was in very poor general condition, with persistently abnormal erythrocyte sedimentation rate and formol-gel reaction. Later he developed pericarditis and was gravely ill.

Case 3.- J.G.J., aged 16 years.

Family History.- Nil relevant.

Previous History.- Nil relevant.

Present History.- Chart 3 (p.237).

30.5.39-31.5.39: Onset of pain in back, both knees, elbows and left ankle.

3.6.39- 5.6.39: Joint pains ceased following salicylate therapy, soft mitral systolic murmur present. Severe epistaxis.

10.6.39: Moderate effusion into left knee-joint. For some days there was pain in joints of upper extremities. Further convalescence uneventful.

11.6.39: The left knee was tapped and 14 c.c.

SECTION K TABLES I, II, III

TABLE I.—REACTIONS IN MM. TO INTRADERMAL INJECTION IN SUBJECT A OF MIXTURES OF RHEUMATIC JOINT FLUID AND SERA, FROM PATIENT J. E. B., AFTER INCUBATION AT 37° C. FOR FOUR HOURS.

	Test				Controls		
	Col. 1 19.1.39 Serum + joint fluid	Col. 2 3.2.39 Serum + joint fluid	Col. 3 23.2.39 Serum + joint fluid	Col. 4 29.3.39 Serum + joint fluid	Col. 5 Joint fluid 1 test dose 0·15 c.c.	Col. 6 Joint fluid 2 test doses 0·30 c.c.	Col. 7 29.3.39 Serum 0·15 c.c.
24 hrs.	20 × 30	10 × 10	5 × 5	5 × 5	20 × 35	30 × 45	5 × 5
48 hrs.	20 × 25	0	0	0	30 × 40	30 × 50	0
72 hrs.	10 × 10	0	0	0	20 × 30	20 × 35	0

TABLE II.—REACTIONS IN MM. TO INTRADERMAL INJECTION IN SUBJECT A OF MIXTURES OF RHEUMATIC JOINT FLUID AND SERA, FROM PATIENT K. B., AFTER INCUBATION AT 37° C., FOR FOUR HOURS.

	Test				Controls		
	Col. 1 26.4.39 Serum + joint fluid	Col. 2 3.5.39 Serum + joint fluid	Col. 3 18.5.39 Serum + joint fluid	Col. 4 8.6.39 Serum + joint fluid	Col. 5 Joint fluid 1 test dose 0·15 c.c.	Col. 6 Joint fluid 2 test doses 0·30 c.c.	Col. 7 8.6.39 Serum 0·15 c.c.
24 hrs.	25 × 25	20 × 25	25 × 25	15 × 15	25 × 30	30 × 40	5 × 5
48 hrs.	25 × 30	25 × 30	25 × 25	20 × 20	25 × 30	35 × 50	0
72 hrs.	25 × 30	25 × 30	25 × 25	20 × 20	25 × 30	30 × 40	0

TABLE III.—REACTIONS IN MM. TO INTRADERMAL INJECTION IN SUBJECT A OF MIXTURES OF RHEUMATIC JOINT FLUID AND SERA, FROM PATIENT J. G. J., AFTER INCUBATION AT 37° C. FOR FOUR HOURS.

	Test				Controls		
	Col. 1 8.6.39 Serum + joint fluid	Col. 2 15.6.39 Serum + joint fluid	Col. 3 22.6.39 Serum + joint fluid	Col. 4 13.7.39 Serum + joint fluid	Col. 5 Joint fluid 1 test dose 0·15 c.c.	Col. 6 Joint fluid 2 test doses 0·30 c.c.	Col. 7 13.7.39 Serum 0·15 c.c.
24 hrs.	10 × 15	15 × 15	10 × 10	5 × 5	15 × 20	25 × 35	5 × 5
48 hrs.	10 × 20	20 × 20	10 × 10	5 × 5	20 × 20	30 × 40	0
72 hrs.	15 × 20	20 × 20	10 × 10	0	20 × 20	25 × 30	0

straw-coloured fluid obtained. Culture tests were all negative. On intradermal injection in subject A, 0.3 c.c. of the fresh fluid produced a reaction of 30 X 40 mm. and the stored fluid was equally effective (Table III,col.6., p.239). In this case, there was evidence of neutralisation in the serum dated 13.7.39 (col.4), but not in earlier specimens.

DISCUSSION

The evidence suggested the presence of some toxic or irritative component in the joint fluid from cases of acute rheumatism, as in all three specimens the cultural tests adopted were entirely negative. The appearance of the erythematous reaction produced by the injection of joint fluid was very similar to the Dick reaction. A single attempted neutralisation test by means of horse antistreptococcal serum was not successful on account of the reaction induced by the serum alone. The erythematous reaction was demonstrable in the human subject and not in rabbits nor in guinea-pigs ; a fact in accordance with the observation that rheumatic fever cannot be transmitted experimentally to lower animals. So far only one subject has been tested for reactivity to these joint fluids, and there have been no untoward sequelae.

As regards the properties of the toxin-like component, little information has been obtained because of the difficulty in working with a fluid of

high protein content. Storage at 6°C., followed by incubation at 37°C. for 4 hours caused some deterioration.

The observation that sera in the later stages of convalescence developed the capacity to neutralise the action of the fluid whereas this did not occur at the outset of illness, gave further support to the hypothesis that a toxin-like component was present. The neutralising action of the sera during convalescence was demonstrated in two of the three cases studied, and its appearance coincided with the clinical improvement of both patients. Case 2 was of much greater severity in that pericarditis supervened and no neutralisation effect was demonstrable at any time the patient was under observation.

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SECTION L

THE FORMOL-GEL REACTION AND ERYTHROCYTE SEDIMENTATION RATE IN ACUTE RHEUMATISM

INTRODUCTION

Gaté and Pappacostas (1920) observed that the addition of formaldehyde to Wassermann-positive sera resulted in the solidification of 85 per cent of specimens, while approximately the same proportion of Wassermann-negative sera failed to react in this way. They suggested that this reaction was due to an abnormal distribution of serum proteins. Holborrow (1922) found that forty-five of sixty-four Wassermann-positive sera gave a positive formol-gel reaction, and also seventeen of eighty-nine Wassermann-negative sera. He concluded that the gelation was due to the direct action of the formalin on the serum proteins, and from experimental work considered it probable that acid-protein produced a gel with formalin, while alkali-protein had not this property. Napier (1921-2) and Spackman (1921) observed a similar phenomenon in kala-azar, but noted a difference in appearance between the gelation in that disease and in syphilis. In

kala-azar the serum became opaque and very firm in consistence, as though inspissated, within a few minutes, whereas gelation was relatively delayed in syphilitic sera, taking up to twenty-four hours to appear, and the gel formed was soft and transparent.

As regards the correlation of the formol-gel test with other biological tests, Napier and Henderson (1931) first observed parallelism with the erythrocyte sedimentation test in kala-azar, but they considered the formol-gel test was inferior for diagnostic or prognostic purposes. Pfeffer (1925) stated that the formol-gel test was positive in a proportion of cases of chronic rheumatism and of tuberculosis of the exudative type. Gibson (1938) applied the test, in parallel with the erythrocyte sedimentation rate, to a series of 100 cases of chronic rheumatism. Using plasma, he found that the formol-gel test was negative in 40 of 42 cases with normal sedimentation rates, and positive in 33 of 34 cases with markedly increased sedimentation rates. Approximately half the cases showing a moderate increase in sedimentation rate were positive. He noted a very close parallelism between the two tests, and considered that it may be of use as an additional pathological criterion of activity in chronic rheumatism. Gibson emphasised that the formol-gel test was less sensitive than the sedimentation test in finding evidence of activity in early cases, and considered that it should be used as a supplementary, but

not substitute test. On the other hand, Schultz and Rose (1939) expressed the opinion that, whereas in various febrile illnesses other than rheumatic fever a close parallelism was demonstrable between the erythrocyte sedimentation rate and the formol-gel reaction, unique results were obtained in rheumatic fever. Early in the course of illness negative formol-gel reactions were frequently associated with very rapid sedimentation rates, while after the development of active carditis positive gel reactions appeared often when the sedimentation rate was reverting to normal limits. They concluded that the formol-gel reaction was of value in determining the presence of active carditis in patients known to be suffering from rheumatic fever.

This section records observations on both tests in a group of young male adults in the various phases of acute and subacute rheumatism.

METHODS

Determination of erythrocyte sedimentation rate

10 c.c. of venous blood was thoroughly shaken with 0.02 gm. neutral potassium oxalate in a screw-cap bottle. The Zeckwer and Goodell (1935) method was adopted in principle. Within three hours of the collection of the specimen a 5 c.c. centrifuge tube, graduated in 0.05 c.c., was filled to the 5 c.c.mark with oxalated blood. The volume of the sedimented

red cells was noted after one hour at room temperature. This result was expressed as the percentage volume of red cells, the percentage sign being omitted- e.g. E.S.R. 60 indicates that the volume of red cells, after one hour, was 60 per cent of the total volume of blood. It should be noted that any increase in this figure is interpreted as an approximation to the normal. This reading is, therefore, the reverse of the more usual method of expressing the sedimentation rate as the length of the cleared plasma column above the sedimented cells.

Haematocrit volume

The graduated tube containing 5 c.c. blood was centrifuged for fifteen minutes at 3,000 r.p.m., and the packed red-cell volume expressed as a percentage of the whole blood volume.

Formol-gel test

The supernatant plasma in the centrifuge tube was then separated for the formol-gel test, the quantitative method suggested by Gibson(1938) being used. The only modification introduced was the reduction to half-volume of the various reagents, the relative proportions remaining the same, as follows:

Tube:	1	2	3	4	5	6	7
Plasma	0.5	0.45	0.4	0.35	0.3	0.25	0.5
Saline	0.0	0.05	0.1	0.15	0.2	0.25	0.0
18% formalin	0.04	0.04	0.04	0.04	0.04	0.04	0.0

The tubes were placed in a Wassermann rack supported at one end so that the long axis of the tubes was nearer the horizontal than the vertical plane. After eighteen hours at room temperature the sloped tubes were returned to the vertical position and the occurrence of solidification noted. The results were expressed as follows:-

1+
2+ Degrees of partial coagulation of undiluted plasma

3+: Complete coagulation of undiluted plasma
4+: " " " plasma diluted 0.9 in 1.
5+: " " " " " 0.8 in 1.
6+: " " " " " 0.7 in 1.
7+: " " " " " 0.6 in 1.
8+: " " " " " 0.5 in 1.

N.B.- The following contractions will be used in the text:

E.S.R: Erythrocyte sedimentation rate

H.V:: Haematocrit volume.

F.G.T.: Formol-gel test.

RESULTS

Before attempting to correlate the E.S.R.,H.V. and F.G.T. results, certain basic data were required. The majority of the subjects studied were aged sixteen to eighteen years, and it was considered desirable to ascertain the average or normal limits of these tests in health for this particular age-group and community. All patients were serially tested at the end of prolonged convalescence when about to be discharged as fit. The distribution of the E.S.R. results at this

SECTION L CHARTS 1 and 2

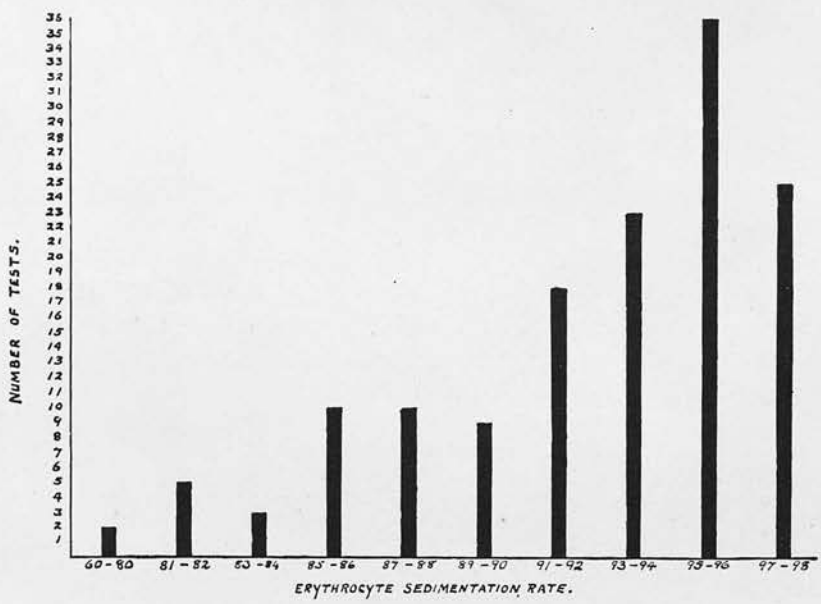

CHART 1.

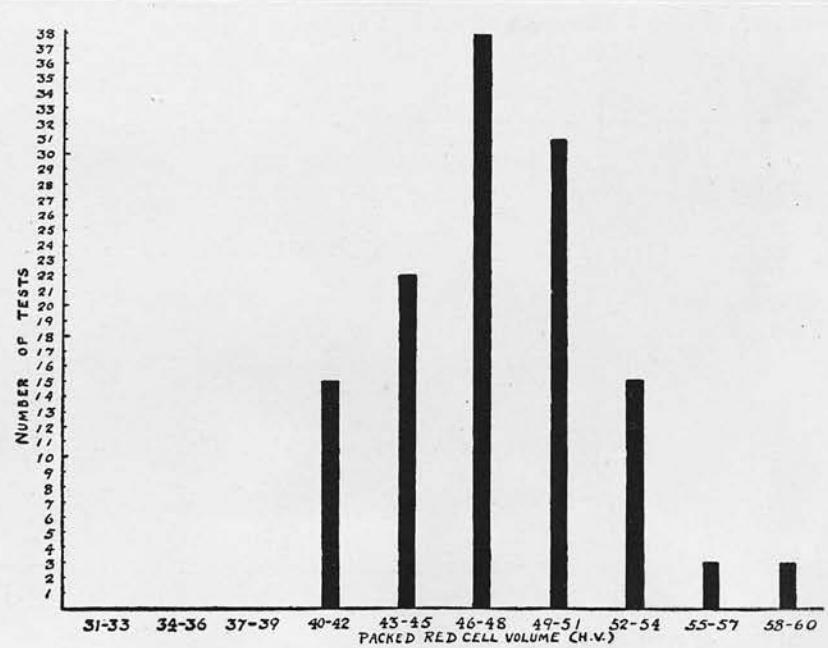

CHART 2.

time is illustrated in Chart I(p.247). Although the majority of the tests upon which this chart was constructed were obtained from rheumatic subjects, a few patients completely recovered from scarlatina and from tonsilitis were included. The series was therefore constituted as follows: 107 tests from 76 rheumatic patients, single tests from 7 cases of scarlatina and from 11 cases of post-tonsilitis patients. Chart I indicates that at the end of convalescence the E.S.R. was most frequently at the 95-96 level, the next largest groups being immediately adjacent to the latter. The average E.S.R., determined by totalling all E.S.R. results and dividing by the number of tests, was found to be 92.4. Table I(p.249)shows even more significantly the high E.S.R. of patients on discharge from hospital, 75.2 per cent of specimens being over 90 and 99.2 per cent over 80. For this reason it was concluded that, although an E.S.R. between 80 and 90 could be considered as possibly abnormal, an E.S.R. below 80 was almost certainly abnormal.

In the same way data on the H.V. of boys on discharge in health were sought. Chart II(p.247)shows that the mean figure was most frequently at the 46-48 level, while the distribution of results on either side of this group fell in the expected manner. The average H.V., determined as above, was 47.9. Table II (p.249) shows that the H.V. of 81.4 per cent of specimens was 45 or higher, and in every case was above 40.

SECTION L TABLES I, II and III

TABLE I.—ERYTHROCYTE SEDIMENTATION RATES OF MALE ADOLESCENTS AT END OF PROLONGED CONVALESCENT PERIOD AFTER ACUTE RHEUMATISM

Erythrocyte Sedimentation Rate.	*Number of Tests.*	*Percentage of ALL Results.*
95 or over	56	44·0
90 ,, ...	94	75·2
85 ,, ...	116	92·8
80 ,, ...	124	99·2
75 ,, ...	125	100·0

TABLE II.—TO SHOW THE HÆMATOCRIT VOLUME OF YOUNG MALE SUBJECTS (95) AT END OF PROLONGED CONVALESCENCE AFTER ACUTE RHEUMATISM

Hæmatocrit Volume.	*Number of Tests.*	*Percentage of Tests.*
55 and over	5	4·0
50 ,, ...	46	37·1
45 ,, ...	101	81·4
40 ,, ...	124	100·0

TABLE III.—TO SHOW THE PERCENTAGE OF POSITIVE FORMOL-GEL REACTIONS IN BLOOD SPECIMENS FROM RHEUMATIC SUBJECTS WITH HIGH, LOW AND INTERMEDIATE ERYTHROCYTE SEDIMENTATION RATES

Erythrocyte Sedimentation Rate.	*Number of Tests.*	*Positive Formol-Gel.*			
		1+ and 2+.	*3+ and over 3+.*	*Total.*	*Percentage.*
100–80	266	4	3	7	2·6
79–60	91	15	31	46	50·6
59–30	70	6	62	68	97·1
Total	427	25	96	121	28·3

All haematocrit volumes lower than 40 were therefore considered to be almost certainly abnormal, and those between 40 and 45 as possibly, but not commonly, in the same category.

Erythrocyte sedimentation rates and formol-gel test in rheumatism

Both tests were applied in parallel in a series of 102 cases of rheumatism in the acute, subacute and convalescent phases. Of this group, 96 cases were boys, aged sixteen to nineteen years, who had been living for some months under similar environmental conditions. The combined results are grouped in Table III(p.249). Only 2.6 per cent of 266 specimens with an E.S.R. over 80 gave a positive F.G.T., as compared with 97.1 per cent of specimens with an E.S.R. lower than 60. Approximately half the cases with an E.S.R. between these limits were positive. In Table IV(p.251) the correlation is indicated in greater detail. Only one positive F.G.T. was found in a series of 173 tests when the E.S.R. was above 90, whereas every E.S.R. below 50 was accompanied by a positive F.G.T. The intervening groups were proportionately related. A further point of interest in the table is that 70.8 per cent of positive F.G. tests were recorded in 161 specimens with an E.S.R. below 80, as compared with 2.6 per cent of 266 specimens in the group above 80. On grouping the positive F.G.T. results at various levels of the E.S.R., as in Table V(p.251), there was

SECTION L TABLES IV, V and VI

TABLE IV.—To show the Correlation between the Erythrocyte Sedimentation Rate and Formol-Gel Reaction in Rheumatic Subjects

Erythrocyte Sedimentation Rate.	*Number of Tests.*	*Positive Formol-Gel.*		
		Number.	*Percentage.*	
100–90	173	1	0·6	2·6
89–80	93	6	6·5	
79–70	51	18	35·3	70·8
69–60	40	28	70·0	
59–50	42	40	95·2	
49–40	27	27	100·0	
39–30	1	1	100·0	

TABLE V.—To show the Mean Formol-Gel Reading of Rheumatic Plasma grouped according to the Level of the Erythrocyte Sedimentation Rate

Erythrocyte Sedimentation Rate.	*Number of Positive Formol-Gel Reactions.*	*Mean Formol-Gel Result.*
100–90	1	1·0
89–80	6	2·1
79–70	18	3·0
69–60	28	3·4
59–50	40	4·2
49–40	27	5·2
39–30	1	8·0

TABLE VI.—To show the Mean Erythrocyte Sedimentation Rate and Mean Hæmatocrit Volume of Blood Specimens from Rheumatic Subjects grouped according to the Result of the Formol-Gel Test

Formol-Gel Test.		*Mean E.S.R.*	*Mean H.V.*
Number.	*Result.*		
9	7+ or more	46·3	38·5
14	6+	51·2	38·6
25	5+	53·8	40·3
23	4+	58·0	39·2
30	3+	66·6	42·4
28	2+ and 1+	67·9	42·7
314	Negative	86·6	44·1

demonstrated a progressive increase in the mean F.G.T. result as the E.S.R. level fell.

Haematocrit volume and formol-gel test in rheumatism

Following the method of comparison used by Gibson (1938), Table VI (p.251) shows the mean E.S.R. and mean H.V. of specimens grouped according to the results of the F.G.T. A reduction in intensity of the positive F.G. reaction was accompanied by progressive increase of the mean E.S.R. and mean H.V. The only exception to be noted in the table was the slightly higher mean H.V. of 40.3 in the 5+ F.G. group as compared with 39.2 in the 4+ F.G. group. Otherwise the correlation was complete. The negative F.G. group included specimens taken in early as well as in late stages of illness, and hence the mean S.R. and H.V. values of 86.6 and 44.1 respectively were lower than those determined at the end of convalescence in the preliminary group, in which the formol-gel test was invariably negative.

Table VII (p.259) shows the percentage of positive F.G. tests at various levels of the H.V. It will be seen that the F.G.T. was positive in 48, or 92.3 per cent, of 52 specimens with an H.V. lower than 40, as compared with 2, or 1.8 per cent, of 113 specimens with an H.V. above 50. Between these two levels, 26.6 per cent of specimens reacted positively to the F.G.T.

Failure in agreement of test results

The instances in which the E.S.R. and F.G.T. failed to give comparable results may now be considered.

E.S.R. within normal limits, F.G.T. positive

Taking the lower limit of normality for the E.S.R. test as 80, 7 specimens were found to give a normal E.S.R., but positive F.G.T.

Case 1.

	E.S.R.	H.V.	F.G.T.	Week of illness.
	86	45	Negative	5th.
First	88	48	Negative	7th.
discrepancy....	94	38	1+	9th.
	94	48	Negative	11th.

This was the only case in which an E.S.R. greater than 90 was accompanied by a positive F.G.T. As seen in the serial record, the F.G.T. was a weak positive.

Case 2.

	E.S.R.	H.V.	F.G.T.	Week of illness.
Second	63	45	2+	8th.
discrepancy....	82	50	1+	10th.
	92	44	Negative	12th.
	82	51	Negative	13th.

From the eighth to the tenth week of illness there was a marked improvement in the E.S.R., but the F.G.T. was still weakly positive during this transition period.

Case 3.

	E.S.R.	H.V.	F.G.T.	Week of illness.
Third	70	40	3+	4th.
discrepancy....	80	44	2+	6th.
Fourth.....	82	44	1+	9th.
discrepancy	82	40	Negative	14th.

Again during the transition period in the patient's progress the E.S.R., while in the limits of normality, was accompanied by a positive F.G.T.

Case 4.

	E.S.R.	H.V.	F.G.T.	Week of illness.
Fifth	79	45	3+	3rd.
discrepancy....	82	50	3+	4th.
Sixth.....	80	48	3+	6th.
discrepancy	90	49	Negative	8th.

Disagreement occurred in the fourth and sixth weeks of a relapse when joint pains and pyrexia were still present. The result of the F.G.T. was a more reliable indication of the clinical condition than the E.S.R.

Case 5.

	E.S.R.	H.V.	F.G.T.	Week of illness.
Seventh	91	50	Negative	1st.
discrepancy...	86	36	3+	2nd.

In this early case stiffness and pain on movement of the leg were the only symptoms present.

In this group, therefore, there was no serious degree of disparity between the results of the two tests.

E.S.R. below normal limits, F.G.T. negative

In 47 specimens with a negative F.G.T. the E.S.R. was below 80. As seen in Table IV p.**251** , 33 of these were in the group with an E.S.R. between 79 and 70, and could be considered as border-line cases similar to those above. The remaining 14 discrepancies of greater degree were as follows:

Case 6.

	E.S.R.	H.V.	F.G.T.	Week of illness.
	94	50	Negative	12th.
First	90	46	Negative	14th.
discrepancy...	58	42	Negative	18th.
	75	44	Negative	20th.

On returning from four weeks' sick-leave, eighteen weeks after the onset of illness, no clinical abnormality was detected to account for the abnormal E.S.R.

Case 7.

	E.S.R.	H.V.	F.G.T.	Week of illness.
Second.....	50	42	Negative	1st.
discrepancy	65	42	3+	2nd.

In this early case there was a definite lag in the appearance of the positive formol-gel reaction.

Case 8.

	E.S.R.	H.V.	F.G.T.	Week of illness.
Third	66	45	Negative	2nd.
discrepancy	88	49	Negative	4th.
	80	47	Negative	5th.
	92	48	Negative	6th.
	94	50	Negative	8th.
	60	42	1+	10th.
	46	37	5+	12th.
	80	42	Negative	15th.

Examined for the first time in the second week of illness, the E.S.R. was definitely abnormal, while the F.G.T. was still negative. The ensuing seven results were in agreement.

Case 9.

	E.S.R.	H.V.	F.G.T.	Week of illness.
Fourth	69	50	Negative	1st.
discrepancy	72	70	5+	3rd.
	56	79	8+	4th.
	58	62	4+	5th.
	60	64	5+	6th.
	66	62	5+	7th.
	52	62	5+	9th.
	62	72	1+	11th.

In this case, also, there was a definite lag in the appearance of the positive F.G.T., but thereafter

the two tests approximated closely.

Case 10.

	E.S.R.	H.V.	F.G.T.	Week of illness.
	62	42	5+	2nd.
Fifth	68	42	6+	3rd.
discrepancy...	64	45	Negative	6th.
	60	44	4+	7th.
	55	42	4+	9th.
	64	45	2+	11th.

After being strongly positive in the second and third weeks the F.G.T. was negative in the sixth week, while the E.S.R. remained at the same low level as in the previous weeks. In the subsequent tests the results were in agreement. No explanation for this discrepancy was discovered.

Case 11.

	E.S.R.	H.V.	F.G.T.	Week of illness.
	96	50	Negative	2nd.
	85	44	Negative	3rd.
Sixth	72	45	3+	4th.
discrepancy.....	65	42	Negative	5th.
Seventh	62	41	Negative	7th.
	73	46	Negative	9th.
	90	45	Negative	11th.

In the fourth week of illness a fall in the S.R. was accompanied by complete gelation of the plasma. In the following four weeks a still further fall in the E.S.R. occurred, but the F.G.T. had returned to normal limits.

Case 12.

	E.S.R.	H.V.	F.G.T.	Week of illness.
	76	48	3+	2nd.
	74	43	3+	4th.
	50	40	3+	5th.
Eighth	65	40	4+	6th.
discrepancy.....	60	40	Negative	7th.
Ninth	62	40	Negative	9th.
	75	40	Negative	11th.
	77	40	Negative	13th.

In this case the F.G.T. had returned to the negative phase at least four weeks before improvement in the E.S.R. began.

Case. 13.

	E.S.R.	H.V.	F.G.T.	Week of illness
Tenth	67	42	Negative	3rd.
discrepancy	77	46	Negative	5th.
	85	50	Negative	6th.
	96	45	Negative	7th.
	96	50	Negative	9th.

This patient was examined for the first time in the third week of illness, when no acute symptoms were present. The E.S.R. was then abnormal, but the F.G.T. was negative.

Case 14.

	E.S.R.	H.V.	F.G.T.	Week of illness.
	50	40	5+	1st.
	46	37	6+	2nd.
	57	35	6+	3rd.
Eleventh	46	38	4+	4th.
discrepancy...	60	50	Negative	5th.
	45	36	4+	6th.
	51	42	3+	8th.
	82	42	Negative	10th.

This disagreement in the fifth week was due to the partial clotting of the blood specimen in the collection bottle. Complete agreement was found at al other times.

Case 15.

	E.S.R.	H.V.	F.G.T.	Week of illness
	74	40	Negative	4th.
Twelfth	70	40	Negative	6th.
discrepancy...	68	39	Negative	8th.
Thirteenth....	60	40	Negative	10th.
discrepancy				

Despite the fall in the E.S.R., the F.G.T. remained negative in the eighth and tenth weeks.

Case 16.

	E.S.R.	H.V.	F.G.T.	Week of illness.
Fourteenth discrepancy....	95	44	Negative	2nd.
	60	45	Negative	3rd.
	79	40	2+	4th.

In this last series of 14 discrepancies, the first two were the only specimens out of 75 with an E.S.R. below 60 which gave a negative F.G.T. The remaining examples were mainly borderline cases occurring at some transition period in the course of illness. These detailed examples otherwise serve to illustrate the correlation which can be expected between these two tests during the course of the infection.

Effect of delay in sedimentation rate and formol-gel test

It is well recognised that there must be the minimum delay in setting up blood for the E.S.R. after the sample has been taken. This may introduce practical difficulties in obtaining results under standardised conditions, particularly if the laboratory be at some distance from the clinical material. For interest, preliminary investigations were made on the relative effect of delay on the E.S.R. and F.G.T. tests. In 105 consecutive specimens both tests were carried out within three hours of collection, as in the routine collection metnod described previously. The E.S.R. and F.G. tests were then repeated twelve and twenty-four hours respectively, after the collection of the blood. As shown in Table VIII(p.259),the sedimentation rate was markedly affected by delay,

TABLE VII.—TO SHOW THE PERCENTAGE OF POSITIVE FORMOL-GEL REACTIONS IN BLOOD SPECIMENS FROM RHEUMATIC SUBJECTS GROUPED ACCORDING TO THE HÆMATOCRIT VOLUME

Hæmatocrit Volume.	*Number of Tests.*	*Positive Formol-Gel.*		
		Number.	*Percentage.*	
30–34	6	6	100·0	92·3
35–39	46	42	91·3	
40–44	135	50	37·0	26·6
45–49	136	22	16·2	
50–54	93	2	2·1	1·8
55–59	20	0	0·0	
Total	436	122		

TABLE VIII.—TO SHOW THE EFFECT OF DELAY ON THE ERYTHROCYTE SEDIMENTATION RATE, AS INDICATED BY THE DIFFERENCE IN READINGS TAKEN WITHIN THREE HOURS AND AFTER TWELVE HOURS OF THE COLLECTION OF BLOOD

Variation in S.R.	*Number of Tests.*	*Percentage of Tests.*	
1–5 increase in S.R.	42	40·0	77·1
5–10 ,, ,, ...	12	11·4	
10–15 ,, ,, ...	14	13·3	
15–20 ,, ,, ...	5	4·8	
20–25 ,, ,, ...	3	2·9	
25 or more increase in S.R. ..	5	4·8	
No change in S.R.	10	9·5	
1–5 decrease in S.R.	14	13·3	

TABLE IX.—TO SHOW THE EFFECT OF DELAY ON THE FORMOL-GEL REACTION, AS INDICATED BY THE DIFFERENCE IN THE RESULTS OF TESTS MADE WITHIN THREE HOURS AND AFTER TWENTY-FOUR HOURS OF THE COLLECTION OF BLOOD

Variation in F.G.T. Result.	*Number of Tests.*	*Percentage.*
1+ increase	5	4·8
No change	85	80·9
1+ decrease	15	14·3

TABLE X.—TO SHOW COMPARATIVELY THE RESULTS OF SCHULTZ AND ROSE AND OF GREEN IN REGARD TO THE CORRELATION OF THE FORMOL-GEL REACTION WITH THE ERYTHROCYTE SEDIMENTATION TEST IN RHEUMATISM

Equivalent E.S.R. Levels.		*Number of Tests at Each Level.*		*Positive Formol-Gel.*			
				Number.		*Percentage.*	
Schultz.	*Green.*	*Schultz.*	*Green.*	*Schultz.*	*Green.*	*Schultz.*	*Green.*
100+	0–50	36	41	13	40	36·1	97·6
70–99	51–65	24	61	16	53	66·6	86·9
40–69	66–80	42	75	25	22	59·2	29·3
20–39	81–90	63	95	23	5	38·7	5·3
10–19	91–95	44	89	7	1	15·9	1·1
0–9	96–100	66	66	2	0	3·0	0·0

there being an increase in the twelve-hour E.S.R. in 77.1 per cent of specimens. In 22 instances an E.S.R. below 80 at three hours was above that level after twelve hours, the increase sometimes being very marked-e.g., 62 to 89, 65 to 93. The formol-gel reaction was found to be much more stable, as Table IX (p.259) indicates. Although the period of delay was twice that introduced in the case of the E.S.R., there was no change in the twenty-four-hour F.G.T. result in 80.9 per cent of specimens. Such variation as did occur was always slight, and never greater than 1+. Only one specimen, initially negative, developed a weak 1+ positive F.G.T. on repetition, and all positive results were confirmed by the second test.

DISCUSSION

It is of great importance that any method of determining the activity of rheumatic infection should be fully investigated, even though the results of the test be non-specific in nature. This investigation supports the conclusions of Gibson (1938) that the formol-gel test is of value in supplementing the erythrocyte sedimentation test. The correlation in results corresponded very closely to those noted by Gibson, and outstanding discrepancies were rarely encountered. This finding is not in accord with the observations of Schultz and Rose (1939). In Table X (p.259), the two series of results are contrasted. It

will be seen that Schultz and Rose noted 36.1 per cent of positive formol-gel tests in specimens with markedly abnormal erythrocyte sedimentation rates-i.e. 100+ Schultz - as compared with 97.6 per cent in the present series. On the other hand, Schultz and Rose found that 18.5 per cent of specimens with an erythrocyte sedimentation rate of 20 or less (Schultz) gave a positive formol-gel test, as compared with only 2.4 per cent in the present series. A possible explanation for these divergent results was the greater frequency of delayed but persistent formol-gel reactions in the series recorded by Schultz and Rose. In the present investigation it was found that, in general, a change from a normal to an abnormal result in one test was synchronous with a similar change in the other. As compared with the sedimentation rate, there may be delay both in the appearance and disappearance of a positive gel reaction, as noted by Schultz and Rose(1939), but the positive gel reaction may precede the appearance of the initial abnormal result in the sedimentation rate.

In its quantitative form the formol-gel test furnished useful information regarding the progress of the individual case. Its utility becomes even greater if there is any possibility of delay in the examination of specimens. The preliminary work has shown that such delay, even up to thirty-six hours after the collection of blood, had little or no effect

on the formol-gel test, whereas any delay beyond three hours or less may completely invalidate the results of the erythrocyte sedimentation rate.

CONCLUSIONS

1. The average erythrocyte sedimentation rate, as determined by the method of Zeckwer and Goodell (1935), and expressed as the percentage red-cell volume, in a group of male rheumatic convalescents aged sixteen to nineteen years was 94.4, and the average haematocrit volume 47.9. The formol-gel test was invariably negative.

2. In 99.2 per cent of cases, on discharge from hospital the erythrocyte sedimentation rate was over 80, and in 81.4 per cent of cases the haematocrit volume was 45 or over.

3. 464 results of erythrocyte sedimentation rate and formol-gel tests on blood specimens from 102 cases of acute rheumatism are compared. Positive formol-gel reactions occurred in 97.1 per cent of specimens with an erythrocyte sedimentation rate below 60, as compared with 2.6 per cent in specimens with rates above 80.

4. In 92.3 per cent of 54 specimens with haematocrit volumes less than 40, the formol-gel reaction was positive.

5. The formol-gel reaction was much less affected by delay than was the erythrocyte sedimentation test.

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GENERAL DISCUSSION

The mass of experimental work conducted in the last few years on haemolytic streptococcal infection has yielded far-reaching results. Although the great advances in the treatment and prophylaxis of disease which followed the introduction of the sulphanilamide group by Domagk (1935) was probably the most out-standing achievement, less spectacular but equally important studies have been made in other fields. In the sphere of epidemiology must be mentioned the work of Griffith (1926, 1927, 1934) for the general adoption of his serological methods made possible the extensive surveys which were previously impracticable. Griffith noted the predominance of types 1, 2, 3 and 4 of Str. haemolyticus in scarlet fever. This distribution was confirmed by Neisser (1939) for strains isolated in the London area, and by Kodama et al. (1939) for strains isolated in Tokyo. Section A of the thesis showed that the distribution was not universal, and that during an epidemic year in Edinburgh other types were commonly found, particularly type 5. Keogh et al. (1939) have similarly reported that types 2 and 17 were recovered from the great majority of scarlatinal patients, and Bailey (1939) has indicated the importance of type 6 in outbreaks in America, although it is

Note

De Waal's work [illegible] a
bearing on Green's and Griffith's type
method - was also used but there was
hardly a continuity of Greens — and
dealt with a different aspect of the problem
the staphylococcal infection

[illegible]

rarely found in England or in Australia.

There are still epidemiological points which remain obscure. For example, the mechanism whereby large-scale epidemics of scarlatina gain momentum is incompletely understood. In section A of the thesis it was shown that such an outbreak in an urban community was initiated by a rapid increase in the number of cases due to one particular type, but was maintained by successive increases in the proportion of cases due to other types. The important observation was made that the age-incidence in the epidemic year was identical with that of non-epidemic years, indicating that the origin of the epidemic was probably associated with some property of the infecting organism rather than any exceptional variation in the affected community. That the immune state of the exposed population can markedly affect the distribution of cases in a semi-closed community was demonstrated in paper 1 of section I.

At the suggestion of the author, the investigations in Edinburgh were continued in Edinburgh by de Waal (1940, 1941). In a less extensive outbreak of scarlatina in the autumn of 1937, the majority of cases were found to be due to type 1, while type 4 was predominant in a mild epidemic in 1938. The complete absence of type 5 in those two years was in marked contrast to its appearance in the heavy epidemic of

1933, although the wave-like action of various types in maintaining the epidemic was then noted. The effect of such waves must be the production of a state of flux in the general-immunity of the population, for the predominance of certain types is not confined to clinical cases but extends to the healthy subjects in the area, as shown by Kodama (1939). An instance of the probable sequence was afforded by the observation of Keogh (1940) that an epidemic of type 2 infection, in a ward of 59 children, produced 16 cases of scarlet fever while 23 contracted type 2 infection without rash. Evidence was obtained that a number of children who failed to develop a rash owed their resistance to a previous type 6 infection which did not produce rashes. Studies similar to these of section A of this thesis, and of de Waal, provide information of greater value regarding the epidemiological relationship between certain infections than is provided by the study of purely general data, such as case incidence and morbidity rates.

With this object in view, the serological examination of haemolytic streptococci from acute rheumatic and control groups was undertaken. The ideal course in section B was the continuation of Griffith typing, but technical difficulties rendered this impossible. The investigation was therefore simplified by the adoption of Lancefield(1933) grouping. The latter afforded adequate information on the main point

in question, namely, the evaluation of the history of recent naso-pharyngeal infection preceding the onset of sporadic cases of acute rheumatism. The results lent considerable support to the relationship between upper respiratory tract infection by haemolytic streptococci and the induction of the rheumatic state.

This subject was then examined from the allergical aspect in section C. In a limited number of quiescent cases, local and general manifestations of the rheumatic syndrome were produced by subcutaneous injections of haemolytic streptococcal endotoxin, and a greater proportion of susceptible subjects to intradermal injections was noted in a rheumatic group as compared with a non-rheumatic group. Studies of this kind could only be expected to provide suggestive information which became of great importance when considered with other facts.

The preceding investigations were based on indirect methods of study and were followed by the use of antistreptolysin O titration as a direct approach. In section D the value of the test was verified by determining the increase in titre produced by known streptococcal infections, such as scarlet fever. The way was then clear for the comparison of groups of rheumatic and non-rheumatic subjects, which were ideal populations for the experimental observations required, in that all the subjects were of the male sex under similar environmental conditions and in the same

restricted age-group. Under these conditions the higher values of the majority of the rheumatic group were certainly significant, although section B indicated that this may have been due to the pre-rheumatic frequency of naso-pharyngeal infection by haemolytic streptococci.. However, it was demonstrated in section E that the titres continued to rise, in many cases for months, after the onset of rheumatic symptoms but in the absence of any detectable foci of streptococcal infection.

At this stage it was considered advisable to record the comparative studies on the antistreptolysin O response of rabbits following experimental infection with haemolytic streptococci. Although an increase in titre was always obtained, considerable variation in the degree of response and in the clinical condition after identical injections of the antigen was noted. Thus, in one animal a fatal septicaemia was produced, whereas in another of the same weight and in the same non-immune state, there was no demonstrable clinical effect and only the feeblest increase in titre. A point of importance was determined in the course of the comparative work, namely, that an increase in titre was obtained only by the injection of the whole organism or of the separated culture filtrate. In further work (unpublished) the injection of haemolytic streptococcal endotoxin has so far failed to elicit any increase in titre in animals sensitised to streptolysin O.

This work is unfinished, but it is practically certain that, in the experimental animal, the continued supply of the antigen is required for the increase or maintenance of high antistreptolysin O titres. On the other hand, very high titres were noted in acute rheumatism (section E) over a period of months, when the cardiac lesions were the only clinical abnormality present.

With this in mind, the post-mortem investigations in section G assumed even greater significance. It is regrettable that this work was carried out before antistreptolysin O titrations were established as a routine, and no opportunity of repetition has presented itself. However, the results of serial titrations have been invariably high in every case of frank, rheumatic pericarditis studied since that time, and these may be considered to constitute a group in whom the evidence of acute infection was most certain at a time remote from the phase of tonsilitis. The evidence obtained from the post-mortem investigations, in which haemolytic streptococci were recovered in heart-valve cultures from nine out of ten cases of acute rheumatism, pointed unquestionably to the endogenous source of the organisms, although the original site from which they were derived could not be stated with certainty. The distribution of positive cultures among cases and controls appeared to indicate that the verrucae were the location of the organisms. These results, although startling in the absence of

positive signs of pyogenic infection, have since been confirmed by Collis (1939) and by Thomson and Innes (1940). Attention has been directed in section H to the similarities in the morphology of certain coccoid bodies observed in one heart-valve, which yielded a positive culture of Str. haemolyticus, and of cocci in the tissues of rabbits which developed haemolytic streptococcal septicaemia after intravenous inoculation with pericardial fluid from rheumatic cases.

The mass of evidence in section I on the epidemiology of acute rheumatism in semi-closed communities placed great emphasis onnthe association with haemolytic streptococcal infection. Thus the earliest appearance of the disease in a training centre followed an epidemic of scarlatina and tonsilitis. Important factors attending the spread of streptococcal infection in such centres were discussed in paper 1. Probably the largest epidemic of acute rheumatism on record was detailed in the second paper, into which there entered the consideration of the economic state of a "depressed" area and the incidence of rheumatism. This question deserves general revision when the opportunity for sociological investigation returns. A report by the Medical Research Council (1927) concluded that poverty itself could not be directly correlated with the incidence of rheumatism, but the survey was limited to three groups of the less fortunate classes. The significant disproportion in the rheumatic incidence in

recruits to the fighting services, as compared with public school boys of the same age, is nevertheless an established fact. Dudley (1926) was of the opinion that the rate of change of population in the two types of institution was mainly responsible for the discrepancy. At the same time it must be remembered that until recently the recruits were largely drawn from the less fortunate classes, which may have accounted in part for their geater tendency to develop rheumatism.

Further evidence that acute rheumatism was a bacterial infection, as well as the possible introduction of a new form of therapy, was sought in the use of convalescent serum in treatment. The results in the preliminary series, reported in section H, were encouraging but no opportunity has been forthcoming to extend the investigation on a scale large enough to give conclusive results. It must be remembered that the initial symptoms of joint pain, tenderness and swelling, when complete rest is instituted, are of relatively mild nature and often self-limiting at the present time. The efficiency of any new therapeutic measure therefore demands the most careful and prolonged investigation.

One interesting observation was a direct outcome of this latter work. In searching for methods of evaluating convalescent sera for therapeutic purposes, the erythematous reaction produced by the intra-
dermal

injection of rheumatic joint fluid was discovered. The bacteriological examination of these fluids had invariably yielded the usual negative result, but the erythematous reaction was the first demonstration of a toxin-like component. It was found that the reaction could not be induced in laboratory animals, which may be a point of importance when their apparent immunity against rheumatism is recalled. Alternative explanations were, firstly, that the reaction represented a non-specific effect. If this were the case, then the neutralising action of the serum, absent in the acute phase of illness and appearing only in convalescence, was a curious phenomenom. Secondly, although the joint fluids were sterile in the ordinary sense, they may have contained a living agent which required growth factors other than those provided in ordinary broth medium or in blood agar.

It is opportune, at this stage, to review the recent work on the virus aetiology of acute rheumatism. The presence of a particulate agent of the nature of a virus in the pericardial and pleural exudates from acute rheumatism was concluded by Schlesinger, Signy, Amies and Barnard (1935) , on the grounds that suspensions of these particles were specifically agglutinated by sera from resistant cases of acute rheumatism, but not by sera from normal persons nor even from closely allied conditions such as rheumatoid arthritis. These

observations were generally confirmed by Eagles, Evans Fisher and Keith (1937) who unsuccessfully tested the infectivity of the suspensions in monkeys. Completely negative results of transmission experiments by Schlesinger and Signy (1938) have since appeared. Van Rooyen, Green and Sclater (1937) were unable to confirm the serological findings reported above, and Eagles and Bradley (1939) have concluded that it was questionable whether the agglutination of particle-suspensions, which they elicited equally well - in approximately 40 per cent of specimens- with sera from rheumatic fever, arthritis of the rheumatoid type of unknown aetiology or arthropathies not classified as true rheumatism, could be accepted as undoubted evidence that these suspensions contained virus elementary bodies. Apart from the evidence of serological and animal transmission experiments, the distribution of acute rheumatism in semi-closed communities (paper 3, section 1) did not conform to that of any of the known virus diseases. The latter were characterised by sharp explosive outbreaks, whereas cases of rheumatism were more uniformly distributed in relation to time. Furthermore, the author has made extensive use of the technique of chorio-allantoic membrane inoculation of the developing egg with entirely negative results. This technique was initially adopted in an attempt to repeat the work reported by Swift and

Brown (1939) on the isolation of pleuro-pneumonia-like organisms from rheumatic exudates. In the following year (1940) the authors themselves withdrew their claims.

The properties of the toxin-like component in the joint fluids were not ascertained on account of the lack of suitable material. The presence of neutralising antibodies in the serum during convalescence indicated its probable antigenic nature. On this account, reference must be made to the description by Coburn and Pauli (1939) of the appearance of a precipitogen in the serum, prior to the onset of acute rheumatism. The nature of the precipitogen was not identified, but it was suggested by the authors, following the work of Hughes (1933) on yellow fever, that it may have represented a secondary antigen, resulting from the delayed interaction of streptococcal products and circulating antibodies. If this were the case, then the precipitogen was unlikely to be related to the agglutination of virus-like particles, for Eagles and Bradley (1939) showed that the latter phenomenon did not run parallel to the antistreptolysin O titre. They found that 31 of 110 agglutination reactions were with sera in which the balance of evidence was against an associated streptococcal infection. A relationship cannot be completely excluded on these grounds, for Todd, Hill and Coburn (1940) have reported that

the antistreptolysin S titre tended to be low when the clinical symptoms were most pronounced during rheumatic attacks, whereas the antistreptolysin O titre was then maximum. Coburn (1940) has emphasised that the low antistreptolysin S response was the only exception in a number of tests which revealed streptococcal activity in rheumatism.

Section L was included in the thesis as a recognition of the valuable assistance afforded by such non-specific reactions as the formol-gel test and erythrocyte sedimentation rate, in correlating clinical activity with serological findings. The insidious onset of cardiac complications in certain cases may baffle the most careful observer, but these two tests served to date the recurrence of activity with very few exceptions. According to Lloyd and Paul (1928) the formol-gel test was directly influenced by the globulin/albumin ratio, and positive results indicated a relative increase in circulating globulin. This finding was rediscovered in acute rheumatism by Bradley (1938) who also stated that there was an absolute increase in the volume of plasma and suggested that there were qualitative differences in the proteins. The formol-gel test, therefore, is a useful routine measure, although its non-specific nature must be realised in the interpretation of results.

Although the association with haemolytic streptococcal infection has been fully demonstrated

in the relevant sections dealing with the throat flora, allergical state, antistreptolysin O titre and epidemiology of acute rheumatism, the relative infrequency of the latter is at once apparent. This discrepancy would appear to indicate that factors other than infection are also involved in determining the distribution of rheumatism. Read, Ciocco and Taussig (1938) found that the frequency of rheumatic manifestations was significantly higher among the near relatives of a series of children affected with rheumatic disease, than among the corresponding relatives of a control series of non-rheumatic children. Gauld, Ciocco and Read (1939) continued similar investigations and suggested that hereditary constitution may play a role in the predisposition to rheumatism. Roberts and Thomson (1934) were of the same opinion, although the incidence of the disease in the brothers and sisters of the series they described was small. Recent work has therefore supported the claim for a strong familial and hereditary tendency to rheumatic fever. Wilson and Schweitzer (1937) go so far as to consider that this tendency is transmitted as a Mendelian recessive character. Other conditions which undoubtedly affect the incidence are those which accompany poverty, such as unsatisfactory housing and dietary. These restrictions limit the incidence of rheumatism to a fraction of the figure for streptococcal infections, irrespective of the mechanism which directly precedes the onset

of rheumatism.

As to the actual nature of this mechanism there is a considerable difference of opinion. One theory postulates that an unknown virus is an essential contributory factor, and the evidence in support of this hypothesis has already been discussed. An alternative theory is that recently summarised by Coburn (1940) in which an abnormal response to haemolytic streptococcal infection is considered to be the differential characteristic of the rheumatic state. This abnormality consists in a delayed, inadequate response to the primary throat infection, with the result that viable organisms remain in accessible tissues after the healing of the primary focus. Concurrently, sensitisation of the individual occurs, with retention of antibody within the cells of the reticulo-endothelial system. Subsequently the surviving organisms release more antigen into the circulation, and either the primary antigen or a secondary derivative is phagocytised by the sensitised cells. Contact of this antigen and residual antibody within the cells gives rise to a reaction which stimulates the release of large quantities of globulin into the circulation. At the same time an intense inflammatory reaction and changes in vascular permeability are induced in the tissues, which then become infiltrated with wandering cells, and so give rise to the typical pathology of acute rheumatism.

The investigations described in the thesis gave strong support to the latter theory with certain reservations. Thus the evidence of streptococcal infection was obtained in the majority of cases and an abnormal response was demonstrated. Furthermore, the survival of haemolytic streptococci in the absence of a pyogenic focus was discovered in the post-mortem studies in sections G and H. The two points upon which further investigation are required were the following. Firstly, there was no evidence of streptococcal infection nor of an abnormal response in a minority of cases of acute rheumatism. Admittedly a similar absence of response was seen in a proportion of cases of streptococcal pharyngitis in non-rheumatic subjects (section E), but Coburn (1936) has stipulated that rheumatic symptoms appeared only when a definite increase in antistreptolysin O titre was produced. Secondly, the toxin-like component in the joint fluid in the stage of acute arthritis finds no place in the sensitisation theory unless it represents a form of secondary antigen.

In conclusion then, it is essential that haemolytic streptococcal infection should be placed first and foremost in all questions relating to the spread and reactivation of acute rheumatism, but the problem of aetiology cannot be considered as finally solved.

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